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FROM JOHAN THRONE HOLST'S INSTITUTE FOR NUTRITION RESEARCH, UNIVERSITY OF OSLO, BLINDERN, NORWAY

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I. Studies on digestive juices calcium
II. Long term experiments on strontium-90 metabolism
III. The accretion and resorption of calcium in the skeleton

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Preface

The present work has been carried out at Johan Throne Holst's Institute for Nutrition Research at the University of Oslo. This laboratory has been engaged in research problems connected with the metabolism of calcium for a number of years. The present publication is a continuation of this line of research initiated by the Head of the Institute, Professor Ragnar Nicolaysen. I am greatly indebted to him for his interest and encouragement, and for devoting so many hours to inspiring discussion, as well as for supplying me with the most excellent working facilities; I want to use this opportunity to express my sincere gratitude to him.

This investigation was originally started as a study of calcium metabolism with special reference to the secretion of calcium with the digestive juices. The work was extended to include an investigation of certain aspects of the metabolism of strontium-90. The techniques and methods are to a large extent identical and have been combined in a special chapter. The experiments were carried out during the period November 1956 – June 1959.

I have received financial support from A/S Freia Sjokoladefabrikks Arbeidsfond for Ernæringsforsking, and from New York Community Trust, for which I wish to express my thankfulness. I am grateful to the Norwegian Defence Research Establishment for financial assistance in the investigations on strontium-90.

My thanks are also extended to Miss Gerd Thune, Miss Berit Gröttum, Mr. Jan Möskeland, and Mr. Kåre Nicolaysen, for excellent analytical and technical assistance, and to Professor Lorentz Eldjarn for helpful suggestions for the preparation of the manuscript.

Oslo December 1959.

Fredrik C. Gran.

General Introduction

Various aspects of the metabolism of calcium have been reviewed by NICOLAYSEN, EEG-LARSEN, and MALM (1953), and NICOLAYSEN and EEG-LARSEN (1953). Monographs have been published by SHERMAN (1947), IRVING (1957), and MALM (1958). The last publication bears special reference to Ca requirement and adaptation in adult men.

The structural and functional aspects of the skeleton are reviewed by McLEAN and URIST (1955), NEUMAN and NEUMAN (1958), and McLEAN and BUDY (1959). These subjects are also treated in two monographs edited by BOURNE (1956) and WOLSTENHOLME and O'CONNOR (1956).

A brief recapitulation of the well-established facts may be apt here. More than 99 per cent of the Ca in the body is found in the skeleton as a hydrated Ca-phosphate of undefined stoichiometry present as tiny, submicroscopic crystals, usually referred to as the bone mineral or the bone salt. The skeleton will normally be in a dynamic state of equilibrium, where the anabolic processes will be balanced by the catabolic processes, usually termed bone accretion and bone resorption. The Ca balance of the body depends on several variables, such as the vitamins of the D group, Ca intake and the availability of the dietary Ca, phosphorus intake, the state of growth, a number of hormones, skeletal diseases, etc. These factors may either act singly or in a complicated interplay.

The absorption of Ca from the intestine is normally well adapted to the requirements of the body; the skeletal saturation with bone mineral and vitamin D are the most important factors in the regulation of the efficiency wherewith Ca is absorbed. Dietary Ca in excess of the needs of the body for maintenance of the Ca balance is excreted in the faeces and in the urine together with endogenous Ca.

The endogenous Ca in the faeces originates from the digestive

juices which are secreted during the digestive processes, and the quantity will vary with the efficiency of Ca absorption, since the digestive juices will be re-absorbed from the gut together with Ca of dietary origin.

Strontium resembles Ca in its chemical properties, and is transferred with this element from the diet into the skeletons of animals and humans. Radioactive Sr will follow the metabolism of stable Sr which is normally present in the diet in only small amounts compared with Ca. The importance of Ca in nutrition makes it more natural to consider the metabolic behaviour of Sr in relation to Ca, although the body does not handle the two elements in an identical way.

Part I

STUDIES ON DIGESTIVE JUICES CALCIUM

Chapter 1

Introduction

The true absorption of Ca cannot be estimated in any given experiment without simultaneous estimation of the digestive juices Ca (DJ-Ca). NICOLAYSEN, EEG-LARSEN, and MALM (1953) and NICOLAYSEN and EEG-LARSEN (1953) discuss the secretion and endogenous excretion of Ca in their reviews on Ca metabolism. In a more recent publication, MALM (1958) discusses this subject with special reference to man. Early work is reviewed by NICOLAYSEN (1934) in his work on the excretion of Ca in dogs. These publications should be consulted for references omitted here, especially with regard to the evidence against a regulated secretion of Ca into the gut during vitamin D deficiency.

WALLACE, SHIRLEY and DAVIS (1951) found that all segments of the intestinal tract in rats participated in the secretion of Ca^{45}. The small intestine played a major role in this capacity. THOMPSON, LEWIS and ALVING (1952) found that Ca was secreted into the terminal six feet of ileum in two patients. SINGER *et al.* (1957) also found that the small intestine played the most important role in the secretion of Ca^{45} into the digestive tract in dogs. These authors claim that the large intestine also played a fairly dominant role in this secretion of Ca^{45}. NICOLAYSEN (1934) had previously found that the colon of dogs does not secrete significant quantities of stable Ca, and this has been confirmed by JOHNSON (1937) in man, and by MOORE and TYLER (1955) in pigs. Ion exchange takes place between the tissues of the intestinal walls and the contents of the lumen without any change in the mass of Ca. This may well explain the discrepancies between the findings in experiments carried out with stable Ca or with Ca^{45}. NICOLAYSEN *et al.* (1953) should be consulted.

The available figures for the volumes of the different digestive juices and their respective concentrations of Ca have been compiled

by MALM (1958) among others. According to these data, the gastric and the intestinal secretions are the main sources for DJ-Ca in man; bile, pancreatic juice, and saliva are of less importance. The rat has rarely been used as an experimental animal in studies of the physiology of the formation of digestive juices.

The secretory mechanisms are discussed by BABKIN (1950) in his book on the digestive glands.

The secretion of gastric juice is a continuous process in the rat according to FRIEDMAN (1943) and KOMAROV *et al.* (1944). GRANT (1941 a) found that the Ca concentration of the gastric juice in dogs depends on the type of stimuli; the Ca concentration decreased proportionally with the pH, and the concentration was higher in pyloric juice than in juice collected from the rest of the stomach. Injections of Ca inhibited both the chemical and nervous phase of the gastric secretion (GRANT 1941 b). BABKIN, KOMAROV, and KOMAROV (1940), and SCHIFFRIN (1942) found a diminished volume of gastric juice collected from stomach pouches in hypercalcemic dogs. This may indicate that the gastric juice Ca is associated with the acidity of the secreted juice rather than with the level of Ca in the serum.

BLANSHARD, ARABEHETY, and GRAY (1959) studied the gastric secretion in rats with pyloric ligations in relation to function of the parathyroid glands and hypervitaminosis vitamin D. In spite of great changes in the serum values of Ca, no changes were observed in the volume or in the Ca concentration of the gastric juice. AUSTIN and MATTHEWS (1927) and PARHON and CAHANE (1932), on the other hand, found that the Ca concentration in gastric juice varied with the level of serum Ca in dogs.

MURDOCK and NASSET (1949) observed a diminished volume of intestinal juice from innervated loops of jejunum in hypercalcemic dogs; however, no Ca determinations were carried out on the juices. GASSILOV (1945) found that the Ca concentration in intestinal juices from dogs were approximately equal to the concentration in blood. MALM (1953) found 14.3 mg % Ca in one sample of juice collected from ileum in one subject. VALETTE and CAVIER (1945) found values ranging from 3 to 6 mg % in intestinal juices collected from ligated, denervated loops of jejunum in dogs. GASSILOV (1945) also found that intravenous injections of Ca caused an increase in the concentration of Ca in

the intestinal juices but a decrease in the volume, which may explain why increases have not been observed in faecal Ca after Ca injections; the main route of elimination is the urine.

BECKS and WAINWRIGHT (1943) found great variations in the human saliva concentration of Ca and in the rate of flow. The Ca concentration decreased somewhat when the rate of secretion was stimulated (BECKS and WAINWRIGHT 1942, BRAWLEY and SEDWICK 1938); however, the total Ca secreted increased. DREISBACH (1959) re-investigated the relationship of the Ca concentration in saliva from rat submandibular gland to the level of Ca in blood plasma. The salivary Ca varied inversely with the rate of secretion; it was, however, directly proportional to the ultrafiltrable Ca concentration in the serum. In dogs, ANDREYEV and PUGSLEY (1933) had previously found that hypercalcemia was followed by a rise of Ca in saliva from the parotid gland in dogs with a permanent fistula.

GREENBERG and TROESCHER (1942) found that the bile constitutes an important route of Ca secretion into the intestine in rats. Twenty per cent of the total faecal elimination of Ca^{45} was derived from the bile.

BALL (1930) found that the concentration of Ca in pancreatic juice from dogs was constant and independent of the rate of secretion in animals stimulated by secretin administration (KOMAROV, LANGSTROTH, and McRAE 1939).

Direct determinations of DJ-Ca in intact animals have not been possible before modern isotope techniques provided the tools. A schematic model for the movements of Ca in the digestive tract is presented in figure 1. The model is very simplified; the digestive tract is here regarded as a unity rather than a complex system of different anatomical structures with different physiological functions. If Ca from one of the sources, either the diet or the body, is labelled with Ca^{45}, the mixing of isotopic Ca with stable Ca from the other source will result in a dilution of the specific activity. The quantitative determination of DJ-Ca, endogenous Ca in faeces, true absorption of Ca, etc. is possible provided that the Ca from the diet and the digestive juices are properly mixed so that they are absorbed from the gut at equal rates. A very small amount of the endogenous Ca in faeces is derived from desquamated epithelial cells from the intestinal wall.

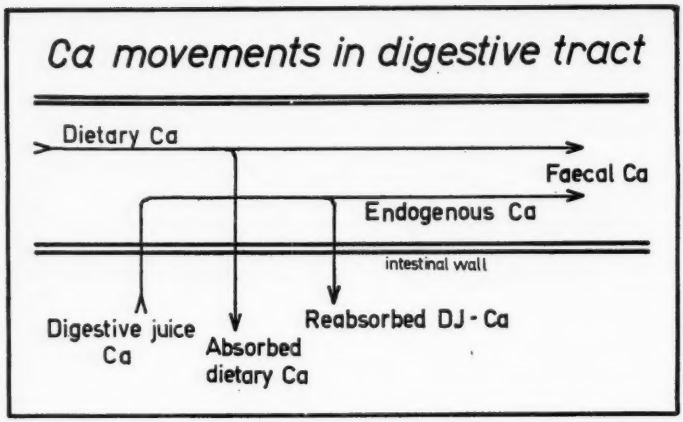

Figure 1.

Schematic diagram for absorption and secretion of Ca in the digestive tract. Dietary Ca is partly absorbed and partly excreted in the faeces together with endogenous Ca, which is the fraction of digestive juices Ca that is not re-absorbed.

VISEK *et al.* (1953) first described a method for the determination of endogenous Ca in faeces from cattle. The specific activities of endogenous Ca and the circulating Ca in the plasma are equal when constant activity in the plasma has been established. This may be accomplished by allowing sufficient time to pass after parenteral administration of the isotope. This method was compared with another procedure by COMAR *et al.* (1953), where the isotope was given orally. In a third report, HANSARD and CROWDER (1957) described a similar technique which corrects for absorbed radioactivity that again is secreted with the digestive juices. The radioactive solution of Ca was given by stomach tube in a single dose, a technique which has been adopted by other workers in the study of the DJ-Ca in man. The amounts of carrier Ca in the dose are in most cases not specified.

The secretion of Ca with the digestive juices and the endogenous Ca in faeces have been reviewed by COMAR (1956, a, b), COMAR and WASSERMAN (1956), and by HANSARD (1956), with special emphasis on the work in their laboratory, which was mainly carried out on cattle. According to these reviews, endogenous Ca in faeces

Table 1. The endogenous Ca in faeces and digestive juices calcium in various species determined with Ca [45]

Species	Age	Body	Calcium	Endogenous	Digestive

Table 1. The endogenous Ca in faeces and digestive juices calcium in various species determined with Ca [46]

Species	Age	Body weight	Calcium intake per day	Endogenous calcium per day	Digestive juices Ca per day	Method	Reference
Rat	12 weeks	103 g	0.8 mg	0.8 mg	40.5 mg	Oral or	HANSARD and
	12 «	171 g	24 mg	5.1 mg	36.4 mg	parenteral	PLUMLEE (1954)
	12 «	171 g	45 mg	7.4 mg	20.0 mg	administration.	
	12 «	198 g	102 mg	19.1 mg	36.0 mg	Single doses.	
	4 «	40 g		1.2 mg	60.0 mg	Oral, corrected	HANSARD and
	12 «	180 g		7.0 mg	16.3 mg	for secretion	CROWDER (1957)
	24 «	310 g		12.0 mg	22.2 mg	of absorbed	
	48-72 «	450 g		18.0 mg	30.5 mg	activity.	
	106 «	450 g		22.0 mg	29.0 mg	Single doses.	
Man	45 years		1.16 g	0.44 g	1.91 g	Oral, single dose	FINK and LASZLO (1957)
	52 «	50 kg	0.53 g	0.09 g	0.14 g	Oral, excretion of	BLAU *et. al.* (1957)
	72 «	67 kg	0.14 g	0.12 g	0.36 g	absorbed dose.	
Cattle	1 week	34 kg	8.8 g	0.41 g	20.5 g	Oral or	COMAR (1956 b)
	1 month	69 kg	7.4 g	0.97 g	48.5 g	parenteral adm.	
	6 months	183 kg	21.0 g	3.3 g	5.6 g	Single doses.	
	Adult	318 kg	27.0 g	5.4 g	8.5 g		
	Adult	438 kg	19.0 g	9.2 g	11.8 g		
	2 months	79 kg	9.1 g	1.7 g	28.3 g	Oral adm.	LENGEMANN, COMAR and
	2 «	62 kg	12.8 g	1.5 g	2.6 g	Single doses	WASSERMAN (1957)
	5 «	132 kg	11.4 g	3.8 g	18.1 g		
	5 «	144 kg	31.9 g	1.4 g	1.7 g		
		253 kg	26 g	2.6 g	3.6 g	Parenteral	COMAR (1956 a)
		350 kg	20 g	2.6 g	3.9 g		
		258 kg	58 g	3.9 g	5.2 g		
		357 kg	43 g	3.4 g	4.4 g		
		397 kg	13.5 g	4.5 g	13.6 g	Parenteral	COMAR *et. al.* (1953)
		258 kg	23.7 g	4.4 g	20.6 g	Parenteral	
		309 kg	55.0 g	5.0 g	56.0 g	Oral, single dose	
		397 kg	13.0 g	4.6 g	12.0 g	Oral, single dose	

remains unchanged during the years of maturity, although it increases in old age. The Ca content of the diet consumed at the time of the experiment has little effect on the endogenous Ca in faeces. During the course of time, adaptive processes are brought into play in the animals fed on a low Ca diet, thereby reducing the endogenous loss of Ca in faeces.

A compilation of the available literature is given in table 1. The methods outlined above have been used with only minor changes. In several of the reports, the figures for the DJ-Ca are not given, and they have therefore been calculated from the other figures reported. Considerable variations are seen in the DJ-Ca in the three species listed. The variations are specially great in man and in cattle, but in the case of the rat the range is not so wide.

Relatively good indirect estimates are available for DJ-Ca in man. MALM (1958) arrives at an estimate of approximately 760 mg per day, with an extreme range of 400 to 1100 mg daily. A few observations in man (ACKERMANN and TORO 1953, BOGDONOFF, SHOCK, and NICHOLS 1953) indicate that Ca in faeces may exceed the intake by more than 800 mg per day. The three values reported for man in table 1 are not in agreement with these findings. The two patients reported by BLAU *et al.* (1957), however, were on a low caloric intake, and suffered from neoplastic diseases in the final stages.

The experiments carried out with rats are probably the best controlled. Accurate balances are easily obtained in this animal, as is probably reflected in a narrower range of values for the DJ-Ca.

Chapter 2

Plan of investigation

As outlined above it is by now well established that Ca is secreted with all digestive juices. The total amount per day is a sizable quantity. It is of general physiological importance to have methods of direct estimate under variable conditions. The exact evaluation of the true absorption of Ca under given experimental conditions is only possible when the digestive juices Ca is simultaneously estimated by direct analysis. The result of a few direct analyses of the daily amount of this parameter agree poorly with the indirect

estimates, and the results in animals with the direct isotope methods indicate very great variability within the same species.

The studies of the separately collected digestive juices, saliva, stomach juice, etc., have resulted in a series of values which were obtained, however, under conditions which differ widely from those under which digestive juices are secreted under normal physiological circumstances in daily life.

The following may serve as a useful summing-up of the observations on the separately collected digestive juices:

1) The amount secreted varies considerably, and the type of stimulus is of primary importance.
2) The concentration of Ca varies and seems to depend upon the rate of secretion.
3) The Ca concentration of plasma seems to influence not only the Ca concentration in the digestive juices but also the rate of flow.

So far no systematic study is available of factors influencing the total digestive juices Ca under physiological conditions.

Thus the plan of the work here presented was somewhat as follows:

1) To work out a dependable method for the determination of the DJ-Ca under normal physiological conditions.
2) To determine the magnitude of DJ-Ca in rats. It was of importance to observe intra- as well as the inter-individual variability. It seemed that repeated studies in the same animals and in sizeable groups of animals were required for the elucidation of such variations. From this point of view long-term balance studies in rats seemed well suited.
3) To determine the total amount of DJ-Ca secreted under varied conditions:
   a) plasma Ca concentration,
   b) the amount of food eaten,
   c) the Ca balance of the body, i. e. the degree of skeletal saturation with Ca, and
   d) the effect of vitamin D.

Chapter 3

Experimental methods

Rats

Hooded and albino rats of both sexes were taken from the strains of the Institute's rat colony. These strains have been interbred in this laboratory for about 20 years. In order to obtain rats which were representative of their age and previous history, the animals were selected by body weight and appearance, excluding those of abnormal size or with other abnormalities.

The age of the rats varied according to the type of experiment. Since the experiments had to be performed under proper control of the vitamin D content of the rats, the animals were fed the pre-experimental diet instead of the stock diet, since the latter contained small amounts of vitamin D. The vitamin D supplemented rats also received this ration (see the following section).

Blood was taken from the tail for determinations of serum Ca while the rats were maintained in slight ether anesthesia. The flow of blood through the cut was facilitated by heating the tail slightly under an infra-red lamp.

Parathyroidectomy was carried out under ether anesthesia by careful cauterization of the two glands, and part of the thyroids was simultaneously removed. The success of the operation was checked by determinations of serum Ca at intervals after the operation; the animals which failed to show a marked fall in the value for serum Ca and to maintain this low value were excluded from the experiments.

At the conclusion of each experiment, the rats were killed by exsanguination under ether anesthesia or after they had been given a stunning blow on the neck.

Diets

The composition of the diets is presented in table 2. Additions of other dietary constituents were made at the expense of wheat flour. The diets were designed to contain approximately 0.25 per cent Ca and 0.4 per cent P; the dietary content of these elements meets the requirements for growing rats (SHERMAN 1947), but better growth may be obtained by additional Ca in the diet. There are several reasons, however, for not raising the dietary levels of

Ca and P, and these will be discussed later (p. 53). The dietary level of Ca was changed in a few instances which will be described in connection with the various experiments.

Table 2. The composition of the diets

Substance	Pre-experimental diet	Diet for determination of DJ-Ca	Diet for experiments with Sr^{90}	Milk-diet
Acid precipitated casein	10.0	10.0	10.0	3.4
Dried whole milk				27.7
Dried egg white (crystalline egg albumen)	3.0	3.0	3.0	3.0
Arachis oil	5.0	10.0	5.0	4.0
Hardened coconut fat (MP 40°C)			10.0	3.1
Dried brewer's yeast	3.0			
Powdered cellulose		5.0	3.0	3.0
Salt mixture, Ca and P free		2.0	2.0	2.0
Sodium chloride	1.0			
Calcium carbonate	0.5	0.5	0.5	
Potassium phosphate, KH_2PO_4	0.8	1.32	1.32	0.76
B-vitamin mixture		0.1	0.1	0.1
Vitamin B_{12} in lactose (20 mg B_{12} per kg)	0.5			
Choline chloride		0.05	0.05	0.05
Wheat flour, 70 per cent extraction		67.5	64.5	52.4
Ground whole wheat	76.2			
Chromic oxide		0.5	0.5	0.5

The figures are in per cent. The pre-experimental diet was the diet of HAAVALDSEN & NICOLAYSEN (1956).

The salt mixture contained 68.8 % potassium chloride, 24.9 % sodium chloride, 2.24 % magnesium chloride, 0.05 % manganese sulphate, 0.09 % copper sulphate, 0.02 % potassium aluminium sulphate, 0.14 % sodium fluoride, 3.84 % ferric citrate, 0.01 % potassium iodide, 0.005 % cobalt chloride, and 0.002 % sodium arsenate.

The B vitamins were also mixed separately before they were added to the diet, and contained 0.8 g thiamine, 1 g riboflavin,

1 g pyridoxine, 5.6 g Ca pantothenate, 2.0 g niacin, 40 g p-aminobenzoic acid, 40 g inositol, 0.02 g biotin, 0.04 g folic acid, 0.002 g vitamin B_{12}, and 109.54 g lactose. In addition, 0.4 g menadione was added to this mixture. One gram of this mixture was added to each kg diet.

The components of the diets were mixed in a machine for one hour, whereafter the radioactive isotopes were added in a water solution followed by additional mixing for 15 minutes. Homogeneous distribution of the isotope was ensured by analyses of batches sampled from different places in the dietary container. The diets were stored in the cold-room at −10° C except for smaller amounts which were to be used during the next few days. Sufficient diet was prepared to last for the whole experiment in each case.

The rats received a weekly supplement of two drops of arachis oil containing 140 I.U. vitamin A acetate; a weekly addition of 70 I.U. vitamin D_2 in arachis oil was given when desired.

It might be thought that adsorption of Ca^{45} or Sr^{90} to other dietary constituents, e. g. cellulose, would interefere with the absorption of these isotopes. However, young rats absorbed almost one hundred per cent of the Ca^{45} and eighty per cent of Sr^{89} in the diet, and Sr^{90} could be completely removed by dialysis of a loose paste made up from the diet, which had previously been treated for a few minutes with 0.1 N hydrochloric acid followed by neutralization to pH 7.0.

The great sensitivity of the intestinal tract to ionizing radiation is of importance in long-term experiments with radioactive isotopes added to the diets. The total dose delivered to the epithelial cells of colon was therefore calculated for the following case: The rats received 6.4 μC Sr^{90}-Y^{90} per kg diet in one experiment that lasted for 103 days (table 16). It was assumed that all dietary Sr^{90} was excreted in the faeces together with 5 per cent of the dietary constituents. In this case it was found that the intestinal tissues of colon received approximately 0.5 rad/day. The actual radiation dose will be lower, however, since the contents of the large intestine also contain water and since some of the dietary Sr^{90} is absorbed. The calculated value therefore represents the extreme situation. Incidentally, the rats in this group gained weight normally. In the case of Ca^{45}, which is a weak β-emitter, the radiation dose to the epithelial cells of the intestine will be much lower.

Balance experiments

The investigations were carried out with conventional balance techniques. The rats were housed in individual metabolic cages which allowed separate collections of urine and faeces without contamination by the diet. Coprophagy was prevented by providing the cages with elevated bottoms of wire screen of 1/2 inch mesh.

Water and diet was given *ad libitum* with one exception. A period of two days was usually allowed before balances were started, to ensure complete removal of intestinal contents originating from the previous diet. In the experiments with Sr^{90}, the balances were started from the beginning of radioactive feeding. The metabolic periods lasted from four to seven days.

In short term experiments a serious error may arise when a collected sample of faeces is not representative for the recorded dietary intake. In a control experiment on a number of rats, with carmine as a marker and chromic oxide in the diet, the measured dietary intake deviated by up to ten per cent from the value calculated from recovered chromic oxide in the faeces in a four-day period.

Chromic oxide was quantitatively recovered in the faeces. The faecal recovery was determined in several experiments where a weighed amount of chromic oxide containing diet was given to the rats, which received a diet without the marker before and after this feeding. The recovery varied between 99.5 and 101 per cent. This method was found to be in good agreement with the results of SCHÜRCH, LLOYD, and CRAMPTON (1950), CREMER and LINGEN (1953), and YOSHIDA and MORIMOTO (1957).

In the experiments to be described, the actual dietary intake in an experimental period was therefore always determined with the aid of analyses of chromic oxide in the collected faeces. The figure thus arrived at for the dietary intake was checked against the figure for the directly measured dietary consumption.

The heavy particles of chromic oxide tend to separate from the lighter fragments when dried faeces are powdered. The total collection of faeces from each period was therefore digested with nitric and perchloric acids to prevent errors from inhomogeneous mixing.

The incorporation of chromic oxide in the diet also facilitates the experimental work, allowing omission of the tedious work of separating the periods with carmine marker. The metabolic periods could also be shortened without reduction of accuracy.

Titanium oxide has been used as a marker by several workers. NJAA, in a personal communication, reports that the filling of the coecum greatly influences the amounts of faeces collected from the rats. No relative accumulation of titanium oxide takes place in the coecum (NJAA 1957), and it seems unlikely that chromic oxide should behave differently in this respect. Incidentally, titanium oxide was tried, but was replaced by chromic oxide because an additional step in the analytical procedure was eliminated.

Analytical methods

Dietary samples, faeces, and urines were digested with nitric and perchloric acids in Kjeldahl flasks. Chromic oxide was oxidized to chromic anhydride by this procedure; great care was taken to prevent losses of chromic anhydride by distillation from overheated flasks. All analytical determinations could be carried out on aliquots from the digested samples after suitable dilutions.

Chromic oxide was determined by measuring the light absorption of sodium chromate after dilution and alkalinization. The readings were taken in a Beckman DU spectrophotometer at 372 mμ. The light absorption followed Beer-Lambert's law at this wave-length.

Calcium determinations were carried out by the precipitation of Ca-oxalate followed by titration with potassium permanganate according to standard methods. The small amounts of perchlorates present did not interfere with the determinations. The results did not deviate by more than 0.5 per cent from the correct value.

Determinations of Ca in blood serum were initially performed according to NORDBØ (1932), but this method was later replaced by a method developed by the present author (GRAN 1960) in which the accuracy was approximately 3 per cent.

Phosphorus was determined according to FISKE and SUBBAROW (1925).

All determinations were carried out at least in duplicate.

Recovery samples and standards were routinely assayed to check for methodological errors.

Isotopes and isotope determinations

Ca^{45} was obtained from the Joint Establishment of Nuclear Energy Research, Kjeller, pr. Oslo, Norway. The activity was usually in the order of 1 mC per 4 g Ca carbonate. Sr^{89} was also obtained as the carbonate from the same source with an activity of 1 mC per 5.7 g. The activities are given at the time of delivery from the reactor. The Radiochemical Centre, Amersham, England, supplied the preparation of Sr^{90}-nitrate used; this was an essentially carrierfree preparation containing 0.1 mg Sr^{90}-Y^{90} nitrates per mC.

The carbonates of Ca^{45} and Sr^{89} were converted to the corresponding chlorides by the addition of hydrochloric acid. The Sr^{90}-nitrate was diluted to volume directly. The isotopes were added to the diets to a convenient activity level (500 to 2,500 cpm per mg Ca.).

The isotopes, or isotope-containing diets were administered to the rats in a room equipped for this purpose. The special safety problems arising from the possible spreading of radioactive dust from the diets led to security measures, such as routine monitorings and dust collections to prevent possible contamination. Great care was exercised in the handling of the isotopes, diets, rats, and various equipment. Rubber gloves and dust masks were always used in this room. The floor and shelves were frequently washed with soap and water.

All isotope determinations were carried out at least in duplicate after the digestion of the samples with nitric and perchloric acids.

The determinations of Ca^{45} were carried out according to the recommendations of COMAR (1955). Four mg of carrier-Ca were added to a suitable aliquot, followed by the addition of ammonium oxalate in excess, and adjustment of the pH to the pink colour of methyl red by the dropwise addition of dilute ammonia or acetic acid. The precipitate was left overnight to allow crystal growth, followed by centrifugation and one washing of the precipitate with distilled water. The precipitate was stirred into suspension and transferred quantitatively to small aluminium

dishes, using small volumes of water. After even distribution of the precipitate had been ensured the solids were allowed to settle, and water was removed by careful evaporation under an infra-red lamp.

Preliminary investigations revealed that Sr^{89}, after the addition of carrier Ca and Sr, could not be quantitatively precipitated as fluoride, carbonate, sulphate, or oxalate. Precipitations with oxalate had to be carried out twice after additions of stable carrier each time, to ensure complete removal of Sr^{89} from the solution. We found that complete precipitation was obtained from 50 per cent ethanol in the following manner: Two mg Sr and four mg Ca were added as carriers to an aliquot of the sample in a centrifuge tube. Two drops of methyl red and 1 ml 20 per cent sodium acetate were added, followed by a volume of 95 per cent ethanol equal to the total volume plus two ml in excess. One ml saturated ammonium oxalate was added, and pH was adjusted to the proper value by the dropwise addition of dilute ammonia or acetic acid. At least two hours were allowed to pass before the tubes were centrifuged. The precipitate was washed once with distilled water and transferred to aluminium dishes in the way previously described.

In order to establish equilibrium between Sr^{90} and Y^{90}, 21 days were allowed to pass after the samples had been prepared before they were counted. The activity was measured in a thin end-window GM tube with a mica thickness of less than 1.8 mg/cm^2. The counting rates were corrected for coincidence losses, background activity, self-absorption, and if necessary radioactive decay. Corrections were not applied for self-absorption in the case of Sr^{89}. A total of at least 3,000 counts were recorded to depress the counting error to less than two per cent. The procedures were checked by preparing curves for self-absorption and isotope dilution. Good agreement was obtained with the self-absorption curve given by COMAR *et al.* (1951) for Ca^{45}. The self-absorption curves for Ca^{45} and Sr^{90} are given in figure 2.

Complete recovery was obtained by the precipitation and plating procedures used for isotope determinations when these methods are compared with directly plated samples.

In the routine determinations of Ca^{45}, Sr^{90}, or Sr^{89} and Ca^{45}, the precipitates could not be washed with dilute ammonia as this

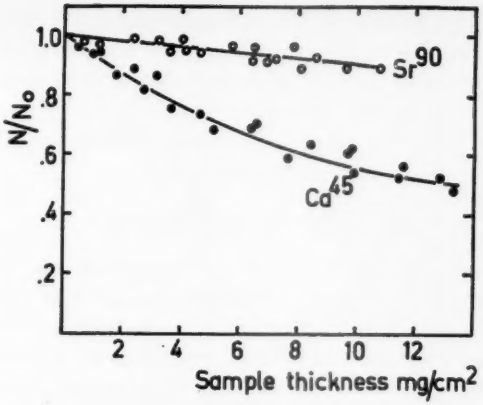

Figure 2.
Self-absorption curves for Ca^{45} and Sr^{90}.

resulted in the formation of hydrogen when the ammonia came into contact with the aluminium of the dishes. No loss of radioactivity was observed when water was used to wash the precipitates.

Sr^{89} and Ca^{45} in mixture were plated in the same way as described for Sr^{90} or Sr^{89}. The plates were counted under a thin end-window tube; a second counting was carried out in the presence of an aluminium filter placed between the tube and the sample. The absorber thickness was 53.6 mg/cm², which completely

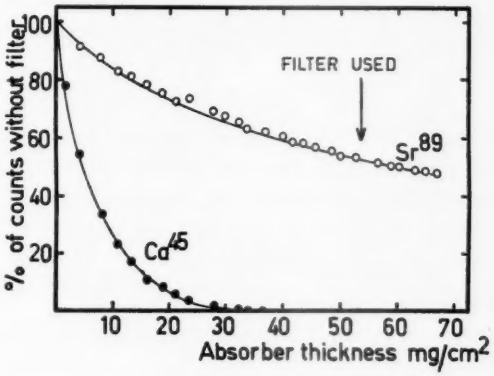

Figure 3.
Absorption curves for Ca^{45} and Sr^{89} using aluminium absorbers.

absorbed the radiation from Ca^{45} and reduced the Sr^{89} activity to 54 per cent of the activity without filter. The procedure follows the principle of COMAR (1955). The absorption curves for Sr^{89} and Ca^{45} are given in figure 3.

Chapter 4

Outline of experiments

The following chapters will describe methods of calculation, general considerations, and selection of a suitable method for the determination of DJ-Ca, followed by the description of one experiment in full detail. In the sections which follow, the experiments in each series have been combined in order to save space and to facilitate a coherent presentation.

Next comes a comparison of the results obtained with a low Ca diet and with a diet with a moderate content of Ca. The effects of vitamin D, the parathyroids, and thyroxin were then studied in order to establish the effect of a variation of the plasma Ca level on the secretion of DJ-Ca. Thereafter, the secretion of DJ-Ca was investigated in relation to the amount of food eaten in normal and vitamin D free rats.

Chapter 5

Calculations

The Ca found in faeces is the composite of the following components:

$$Ca_f = Ca_i - Ca_a + Ca_s - Ca_{sa} \qquad \text{(Eq. 1)}$$

where Ca_f is the faecal Ca, Ca_i is the ingested Ca, Ca_a the amount of ingested Ca absorbed, Ca_s the digestive juices Ca, and Ca_{sa} re-absorbed DJ-Ca; see figure 1.

Endogenous Ca (Ca_e) in the faeces is the fraction of digestive juices Ca which is not re-absorbed.

The absorption coefficient is defined by:

$$X = \frac{Ca_a}{Ca_i} \qquad \text{(Eq. 2)}$$

Equation 1 may then be written:

$$Ca_f = Ca_i + Ca_s - X(Ca_i + Ca_s) \qquad \text{(Eq. 3)}$$

on the assumption that the rates of absorption are equal for Ca derived from the diet and the digestive juices.

The absorption coefficient X cannot be obtained in experiments with inactive Ca only. However, with the aid of Ca^{45}, X can readily be calculated, when the isotope is given in the diet:

$$X = \frac{Ca_i^{45} - Ca_f^{45}}{Ca_i^{45}} \qquad \text{(Eq. 4)}$$

The digestive juices Ca is the only unknown in Eq. 3, and is readily calculated from the re-arranged equation (cf. NICOLAYSEN, EEG-LARSEN and MALM 1953):

$$Ca_s = \frac{Ca_f}{1 - X} - Ca_i \qquad \text{(Eq. 5)}$$

It has been assumed in the above that the specific activity of the digestive juices Ca is negligible. The concentration of Ca^{45} in the blood plasma will increase, however, since some isotopic Ca is absorbed. The amount of endogenous Ca^{45} (Ca_e^{45} below) in faeces will therefore increase in the course of an experiment. The observed value for X will be too low, and an error is introduced in the estimate.

The body handles Ca^{45} and Ca identically. The various secretory glands are supplied from the blood; the specific activity of Ca in the secretory juices and the blood plasma must therefore be equal.

The total radioactivity found in the faeces is given by:

$$Ca_f^{45} = Ca_i^{45} - Ca_a^{45} + Ca_e^{45} \qquad \text{(Eq. 6)}$$

where:

$$Ca_e^{45} = Ca_e \cdot (S.A._{plasma}) \qquad \text{(Eq. 7)}$$

$S.A._{plasma}$ denotes the specific activity of the blood plasma Ca, and $Ca_e = (1 - X)\, Ca_s$.

The following substitutions in Eq. 6 are next conducted:

1) $(1 - X) \cdot Ca_s \cdot (S.A._{plasma})$ for Ca_e^{45}

2) According to Eq. 5, $Ca_s = \frac{Ca_f}{1 - X} - Ca_i$

3) $Ca_a^{45} = X\, Ca_i^{45}$ (see Eq. 2)

which results in:

$$Ca_f^{45} = Ca_i^{45} - XCa_i^{45} + (1 - X)\left(\frac{Ca_f}{1 - X} - Ca_i\right)(S.A._{plasma})$$

The equation is solved for X:

$$X = \frac{Ca_i^{45} - Ca_f^{45} + (Ca_f - Ca_i)\ (S.\ A._{plasma})}{Ca_i^{45} - Ca_i\ (S.\ A._{plasma})} \qquad \text{(Eq. 8)}$$

from which the correct value for Ca_s can be calculated according to Eq. 5.

Reference should be made to the general discussion, p. 51, for comments on the sources of errors in the calculated results. It is pertinent already at this point, however, to point out that since X is the result of three different countings (Eq. 8), an error in the calculated value for X is mainly caused by the counting errors. It is therefore very important to conduct the experiments in such a way that the value for X is kept low (see footnote on p. 51) in order to reduce the error in the calculated DJ-Ca.

Chapter 6

General considerations

It follows from the preceding chapter that it would be beneficial to keep the absorption of Ca at its lowest possible value, at least in the initial phase of this work, before sufficient experience had been obtained with regard to the method, etc. Oxalate added to the diet will repress the absorption of Ca and the secreted DJ-Ca will be trapped in the intestine.

The ingestion of high Ca diets, however, will result in a smaller percentage of absorption of food as well as of DJ-Ca. In consequence the variations in the faecal Ca^{45} will be reduced, and considerable errors may occur from small errors in the analyses. No work has been carried out with high Ca diets for this reason.

It seemed to be of advantage to work with rats with inherent low rate of absorption due either to body saturation with Ca or to vitamin D deficiency. Vitamin D free rats were therefore used initially to establish the method to be used in the investigations.

A priori a correlation between plasma Ca concentration and the amount of DJ-Ca seemed probable on the basis of the experiments quoted. It was therefore an important part of the plan of work to establish conditions in which sizable variations in plasma Ca occurred. In recent years considerable attention has been paid anew to the influence of vitamin D on plasma Ca, and it has been

established that vitamin D is an important factor in the Ca homeostasis (CARLSSON 1952, NICOLAYSEN and EEG-LARSEN 1956). In the experiments presented here, adult rats were used. The above-mentioned homeostatic effect was established in very young rats only.

In the course of the present experiments a number of observations on the slow development of low plasma Ca values in adult, vitamin D free rats were made, and a brief description of these is appropriate. Tables 3 and 4 are instructive. It is seen that in the 13-month-old rats about ten months of vitamin D deprivation is required to reduce the plasma Ca by one-third. In the 24-month-old rats the development was much more variable (table 4). The course of the plasma Ca reduction is thus not fully predictable, and in the experiments presented later in this work selections were made following plasma Ca analyses in each vitamin D free rat.

The status of Ca absorption was always checked in pre-experimental, metabolic periods, in which the rats were given a diet comparable to the pre-experimental diet with respect to the contents of Ca and P. Rats which showed anomalies were discarded.

Table 3. Serum calcium in vitamin D free rats

Age at time of sample Months	Serum-Ca mg/100 ml
13	10.0 ± 0.2
15	8.6 ± 0.1
16	8.9 ± 0.2
18	8.7 ± 0.2
19	8.2 ± 0.3
22	8.8 ± 0.5
24	7.0 ± 0.2

Mean ± standard error of the mean.
Ten female rats received stock diet until they were 13 months old. Next the pre-experimental diet was given with 0.25 per cent Ca and 0.4 per cent P. Samples of blood for determinations of Ca were taken from the tail at intervals indicated above. Vitamin D was not given.

Table 4. Distribution of values for serum Ca in old rats

Number of rats	Range of serum-Ca mg/100 ml
5	5.0—6.9
5	7.0—7.9
8	8.0—8.9
7	9.0—9.7
Average for 25 rats	8.0±0.2

The rats were 28-month-old females which had been deprived of vitamin D during the previous four months. The pre-experimental diet containing 0.25 per cent Ca and 0.4 per cent P was given.

Chapter 7

Choice of method for the determination of DJ-Ca

The selection of a suitable and reliable method for the determination of DJ-Ca in rats was obviously a prerequisite for the experimental work. As previously mentioned, it was desirable to employ a method by which successive determinations of DJ-Ca could be carried out in the same rat. The methods of VISEK *et al.* (1953) and HANSARD and CROWDER (1957) were tried; in addition a third method was studied by which the isotopic Ca was administered by continuous ingestion with the diet.

A. Determination of DJ-Ca by isotope dilution

The method of VISEK *et al.* (1953) appears to be theoretically sound on one condition. The principle of the method is based on the establishment of a constant plasma specific activity with the aid of repeated parenteral injections, and the precision of the estimation of the specific activity of plasma and faeces. The actual formula for the calculation is:

$$\frac{\text{Specific activity faecal Ca}}{\text{Specific activity plasma Ca}} \cdot \text{faecal Ca} =$$

endogenous Ca in faeces on any given day of observation.

One preliminary experiment was carried out in order to determine the applicability of the method of VISEK *et al.* (1953). A group of four rats on seven successive days received one subcutaneous injection each of Ca^{45}-chloride in saline. The rats were 14 months old and free of vitamin D. For two months prior to the experiment these rats had been maintained on a diet with 0.25 per cent Ca and 0.4 per cent P. At the start of the experiment they were changed to a low Ca diet (0.026 per cent Ca) with 0.5 per cent ammonium oxalate added.

In rats, the specific activity in serum cannot readily be determined repeatedly unless very high specific activities are used. This experimental difficulty can be by-passed, however, by determinations of the specific activity in the urine which is equal to the average specific activity in the blood plasma during the period of collection (GOEVARTS 1949).

The specific activity of urine and faeces was determined at weekly intervals during the four weeks after the isotope injections had been discontinued. The specific activity in the urine decreased in the course of experiment, and the specific activity in the faeces was higher than or equal to that in the urine, due to the time lag between the actual secretion of the digestive juices and the excretion of the faeces. This error may be reduced by allowing for a lag in the collection of faeces. However, it is impossible to collect correctly the faeces formed and excreted in one period corresponding to the urine excreted in this period.

The implication is that the method is suitable for large animals only, in which the plasma specific activity can be analyzed at sufficiently short intervals. It is possible that the method may also be applied to small animals with a better result if the dietary Ca is increased. However, further work was not continued along this line.

B. Determination of DJ-Ca by isotope ingestion in a single dose

HANSARD and CROWDER (1957) described a method by which Ca^{45} was administered to the rats in a single dose by stomach tube. Corrections for absorbed radioactivity which re-appeared in the faeces were applied, using the values from identically treated

rats which had received the isotope by injection. It was desirable to compare this method with other methods.

A total number of 30 vitamin D free rats, approximately one year old, were divided into four groups. Each group received a diet containing 0.84, 0.43, 0.23, or 0.13 per cent Ca, respectively. After an initial period of four days, half of the rats in each group were given 4 ml Ca^{45} by stomach tube after they had been fasted for five hours. The dose administered contained 1.9 mg Ca and 109,000 cpm Ca^{45}. The stomach tube was rinsed with one ml water to wash down the radioactive dose. For activity determinations the same dose was measured into a volumetric flask in a manner identical to the way in which the dose was given to the animals. The remaining rats in each group received the same dose by subcutaneous injection of the Ca^{45} solution made up in 0.9 per cent sodium chloride. All rats were maintained in slight ether anesthesia while they were handled.

The rats were allowed to continue on their respective diets for four days. Faeces were analyzed for Ca and chromic oxide and the recovery of Ca^{45} was determined. The results are found in table 5.

Additional experiments were carried out with 20 male rats, seven months old, which had previously been given the pre-experimental diet over four months with supplement of vitamin D. These rats were divided into four groups which were given diets with 0.84, 0.43, 0.23, or 0.13 per cent Ca, respectively, for sixteen days. The animals were given 3 ml of a carrier-free solution by stomach tube after four hours fasting, followed by one ml of water as described for Ca^{45}. The activity determinations of the dose received were carried out on an identically measured volume in this case also.

The rats were kept on their respective diets for five days. Faeces were analyzed for Ca and Sr^{90} as well as chromic oxide. The results are given in table 6.

The serum Ca varied with the level of Ca in the diet in table 5. This result is in agreement with the well-known fact that serum Ca in the vitamin D free rats will also be influenced by the level of Ca in the diet. Endogenous Ca and DJ-Ca were calculated according to HANSARD and CROWDER (1957). Great variations are seen as would be expected, since the recoveries of orally given doses were almost identical in the different groups, which was also the case in the experiments where Sr^{90} was given to rats supplemented

Table 5. The determination of digestive juices Ca by administration of Ca^{45} in a single dose

Ca in the diet	Body weight	Dietary intake	Ca-intake	Ca in faeces	Percent of orally administered Ca^{45} in faeces	Percent of Injected Ca^{45} in faeces	"Endogenous" Ca	"Digestive juices Ca"	Serum Ca
Per cent	g	g/day	mg/day	mg/day			mg/day	mg/day	mg/100 ml
0.840	267	8.7	72.8	83.9	78.6	29.8	26.6	33.9	10.1
0.430	238	9.6	47.8	48.3	72.8	27.1	11.1	15.2	7.8
0.228	236	12.8	29.3	29.4	81.5	27.6	5.5	6.7	7.6
0.127	245	12.8	16.3	18.2	78.1	20.9	5.5	7.0	7.3

This study included 30 vitamin D free female rats approximately one year old. The rats were divided into four groups and were kept on their respective diets for four days before 4 ml Ca^{45} (1.9 mg Ca, 109,000 cpm) was given, either by stomach tube to half of the group, or by subcutaneous injection to the rest of the rats. The excreta were collected over the 4 days which followed the administration of the isotope.

Table 6. The retention of a single dose of Sr^{90} given by stomach tube to rats maintained at different levels of Ca intake

Calcium in diet per cent	Body weight g	Calcium intake mg/day	Calcium in faeces mg/day	Sr^{90} in faeces % of dose	Sr^{90} in urine % of dose	Sr^{90} retained % of dose
0.127	305	18.7	15.4	80.9	7.2	11.9
0.228	286	38.0	34.3	86.6	3.8	9.6
0.430	317	68.0	61.8	84.3	4.7	11.0
0.840	352	140.7	133.4	88.4	3.6	8.0

8 mμC (19,000 cpm) in 3 ml of an essentially carrier free solution of Sr^{90}-Y^{90} nitrates (0.9 μg Sr^{90}) was given by stomach tube to each rat after the animals had been starved for five hours. Five male rats, 7 months old, were used in each experiment. The animals were kept on their respective diets for 16 days before the dose was given and for 5 days afterwards while the excreta were collected. 70 I. U. vitamin D was given weekly.

with vitamin D (table 6). The level of Ca in the diet was therefore decisive for the results in DJ-Ca.

It is quite obvious that gross errors are introduced in short-term experiments and it is in fact difficult to conceive of one which would be a true counterpart of the continuous ingestion technique used in the experiments described in the following chapters. As a first approach, the carrier Ca would in amount have to be within the range of the normal consumption of the experimental animal or subject. Next it would be necessary to administer the dose several times a day so that the single load corresponded to the single load of Ca in a meal. However, one would still not have the normal stimuli to digestive juice secretion, and in consequence DJ-Ca would not run normally. The complex effect of the chyme in the stomach and the intestine would still be missing. Other reflections of the same negative type can readily be conceived.

A careful perusal of the works of COMAR and his colleagues indicates that no attention has been paid to the effect of dilution of Ca^{45} with stable Ca. Only tracer doses of Ca^{45} have been used, though occasionally figures for the number of mg Ca injected or ingested are given. Some results, notably with Sr-isotopes, can readily be explained by a failure to obtain complete mixing of

the single oral dose with the contents of the stomach and the intestine. In other experiments with Ca^{45} (HANSARD and PLUMLEE 1954), the results are in line with the expectations, but the reliability of the figures obtained may be dubious.

C. Determination of DJ-Ca by continuous ingestion of the isotope

Next a method which implies continuous ingestion of the isotopic Ca was tried. Four female rats were selected on the basis of comparable body weights and values for serum Ca. The rats were 24 months old and had been deprived of vitamin D for the last eleven months, during which they had received the pre-experimental diet (table 2). Prior to the actual experiment, the Ca absorption was studied for two consecutive periods of four days each. A diet identical in composition to the pre-experimental diet was used with 0.5 per cent chromic oxide added. The rats were found to absorb about four mg Ca daily, a satisfactorily low level.

The rats were next transferred to a diet with 0.058 per cent Ca, 0.4 per cent P, and with Ca^{45} added to the diet. To this diet was added 0.5 per cent ammonium oxalate. It was given two days ahead of the actual balance experiments.

The rats were studied over 32 days subdivided into eight four-day periods. The results are given in table 7. Faecal Ca exceeded ingested Ca by approximately five mg daily. The true absorption of Ca^{45} was found to be about 40 per cent following the correction for the increasing activity of Ca^{45} in blood plasma (see figure 4). The Ca^{45} recovery in the faeces and the specific activity in the urine (figure 4) increased in the course of the experiment. The calculated figures (see page 28) for endogenous Ca in faeces and the DJ-Ca varied from 5.6 to 8.2 mg per day and from 8.5 to 12.8 mg per day, respectively.

In table 8 the results for DJ-Ca are presented for each rat. The variations between the rats and between the periods was considerable and irregular when the single results are compared. When the averages for the four rats are compared, the results deviate only about $\pm$ 15 per cent.

Table 7. The determination of digestive juices Ca. Mean values from a typical experiment

Period No.	Weight start g	Dietary intake g/day	Calcium intake mg/day	Calcium in faeces mg/day	Calcium-45 in faeces Per cent of dose Directly	Corrected	Endogenous Ca in faeces mg/day	Digestive juices calcium mg/day	mg/100 g diet eaten
1	237	7.4	5.0 ± 0.3	8.3 ± 0.4	54.6	—	5.6 ± 0.2	12.8 ± 0.2	119.2 ± 7.7
2	231	9.7	5.6 ± 0.2	10.0 ± 0.4	61.0	57.3	6.8 ± 0.3	11.8 ± 0.7	121.1 ± 8.0
3	229	8.7	5.1 ± 0.8	9.5 ± 0.6	75.6	70.0	6.0 ± 0.3	8.5 ± 0.6	96.8 ± 20.8
4	227	9.2	5.3 ± 0.5	11.8 ± 0.8	78.5	67.6	8.2 ± 1.1	12.1 ± 1.3	131.9 ± 3.8
5	226	9.1	5.3 ± 0.6	11.1 ± 1.0	81.1	70.0	7.3 ± 0.8	10.4 ± 1.4	114.1 ± 10.8
6	221	10.5	6.1 ± 0.3	11.5 ± 0.6	74.4	63.8	7.7 ± 0.4	12.0 ± 0.8	114.6 ± 8.4
7	223	10.8	6.3 ± 0.8	10.5 ± 0.9	74.2	63.4	6.3 ± 0.5	10.0 ± 0.8	92.1 ± 9.0
8	223	9.2	5.3 ± 0.4	9.9 ± 0.6	81.4	70.9	7.8 ± 0.5	11.0 ± 0.7	120.2 ± 3.7

The data are averages ± standard error of the mean.
The corrected isotope recovery in faeces has been corrected for activity in serum of Ca^{45}.
Four female rats were used, about 24 months of age at the start of the experiment. The animals received the pre-experimental diet with no vitamin D in the eleven months preceding the experiment. The experimental diet contained 0.058 per cent Ca, 0.4 per cent P, and 0.5 per cent ammonium oxalate. The specific activity of Ca was 533 cpm/mg Ca. Each experimental period lasted four days. Serum Ca was 8.8 ± 0.9 mg/100 ml at the start and 7.4 ± 0.5 mg/100 ml at the end of the experiment.

Errata

p. 38, table 7. In period 1 the correct figures for dietary intake, calcium intake, and calcium in faeces should be 10.8 g, 6.2 mg, [illegible] respectively instead of 7.4 g, 5.0 mg and 8.3 mg.

Table 8. The secretion of digestive juices Ca. Single observations, periodic averages and rat averages showing the results from a typical experiment

Period number	Rat number: 1	2	3	4	Mean of the period
1	—	12.5	13.2	12.7	12.8 ± 0.2
2	12.7	10.8	13.6	10.1	11.8 ± 0.7
3	—	9.9	8.3	7.5	8.5 ± 0.6
4	16.1	12.1	9.4	10.5	12.1 ± 1.3
5	14.3	9.7	9.3	10.2	10.4 ± 1.4
6	12.8	10.4	14.4	10.5	12.0 ± 0.8
7	12.1	10.8	7.9	11.0	10.0 ± 0.8
8	11.5	10.7	13.4	8.5	11.0 ± 0.7
Average:	13.3 ± 0.8	10.9 ± 0.3	11.2 ± 0.8	10.1 ± 0.5	11.2 ± 0.4

The values are in mg per day ± standard error of the mean.
For balances etc., see table 7.

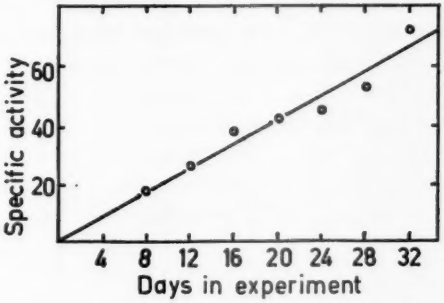

Figure 4.

The specific activity of Ca in the urine from rats following a continuous ingestion of Ca^{45}. The rats were vitamin D free and about 24 months old when the experiment started. Table 7 should be consulted for further details.

The method appeared to be satisfactory. The sources of error which may occur are discussed on page 30, and in the general discussion, p. 51. This method was adopted for the continuation of the work.

The standard error of the mean was calculated according to the formula:

$$s = \sqrt{\frac{\Sigma\ (\bar{x} - x)^2}{n\ (n - 1)}}$$

Errata
p. 38, table 7. In period 1 the correct figures for dietary intake, calcium intake, and calcium in faeces should be 10.8 g, 6.2 mg, [illegible] respectively, instead of 7.4 g, 5.0 mg and 9.3 mg.

Chapter 8

The level of Ca intake and DJ-Ca

Theoretically, the DJ-Ca should not be affected by the level of Ca in the diet at the time of experiment. The next important step was therefore to establish if the method described was applicable when the level of Ca in the diet was raised above that used in the preceding experiment. This was of importance in order to confirm the general applicability of the method, especially with regard to the extension of the work to include rats which were supplemented with vitamin D. It was also of considerable interest to investigate if the oxalate trapping of DJ-Ca was a necessary precaution, since this addition limited the method only to the cases where the absorption could be depressed.

Experiments

The dietary level of Ca was therefore raised to 0.245 per cent in the following experiment and oxalate was omitted in the diet. The composition of the diet was identical in all other respects to the diet previously used in the experiments given in table 7. Six vitamin D free rats were used; the animals were comparable to those used in the experiment given in table 7. The experiment was conducted over three metabolic periods, each of four days duration.

Results

The averages obtained in the experiment with 0.245 per cent Ca in the diet are given in table 9. The results are compared with the averages taken from table 7, where a dietary level of 0.058 per cent Ca plus oxalate was used.

Faecal Ca exceeded ingested Ca by an average of 5 mg daily in the rats given the low Ca diet with oxalate added; the rats on the higher level of Ca in the diet absorbed on an average about 2.5 mg Ca daily. It appears that although the faecal excretion of Ca varied by a factor of almost three between the two experiments, the DJ-Ca was but little influenced by the dietary level of Ca; in fact the figures for DJ-Ca are nearly identical in the two experiments. It appears that the omission of oxalate did not affect the results.

Table 9. The determination of digestive juices calcium in rats on a low or a moderate calcium diet

Per cent Ca in diet	Age months	Body weight g	Number of observations	Serum Ca mg/100 ml	Per cent Ca^{45} absorbed	Ca-intake mg/day	Ca in faeces mg/day	Digestive juices Ca mg/day
0.058	24	231	30	7.4 ± 0.5	36.6 ± 0.5	5.5 ± 0.2	10.4 ± 0.3	11.2 ± 0.4
0.245	24	239	18	8.2 ± 0.6	31.3 ± 0.6	31.3 ± 2.4	28.8 ± 2.3	11.6 ± 0.6

The animals given a low Ca diet with oxalate have been reported previously in tables 7 and 8.
Six comparable rats were given the moderate Ca diet with 0.4 per cent P and Ca^{45} added to the diet.
Both groups of rats were vitamin D free; the serum Ca values are the figures obtained at the end of the experiments.

The serum Ca remained unchanged in the course of the experiment in the group which received 0.245 per cent Ca in the diet, but dropped from an initial figure of 8.8 mg/100 ml to 7.4 mg/100 ml at the end of the experiment conducted with the low Ca diet (table 7). In view of the variations in the DJ-Ca with plasma Ca which are to be discussed in the following chapter, a few comments are necessary. If the average figure for serum Ca for the group on the low Ca diet is taken, the figure is in agreement with the figure for serum Ca found in the group on the higher level of Ca intake. On the other hand, the figures for DJ-Ca in table 7 are more or less constant during the experiment, which may indicate that the low serum Ca level found at the end of the experiment was established only shortly before the experiment was terminated. On this assumption, the serum Ca should also have been comparable to that of the rats on the higher Ca intake.

Comments

It appears from this comparison of rats on two different levels of Ca intake that the DJ-Ca was identical in the two cases. The dietary level of Ca was therefore raised to 0.25 per cent in the experiments to follow because this level of Ca in the diet was of advantage in the analytical work. The figures which were obtained on the lower level of dietary Ca have been included, however, in the averages for DJ-Ca in the following tables.

Chapter 9

The plasma Ca level and DJ-Ca

A priori a correlation between the plasma Ca concentration and the amount of DJ-Ca seemed probable on the basis of the experiments quoted in the introduction. It was therefore an important part of the plan of work to establish conditions in which sizable variations in plasma Ca occurred.

The plasma level of Ca may be changed by several procedures. Vitamin D deprivation will reduce plasma Ca. A further reduction is obtained by the removal of the parathyroid glands.

No experiments were carried out in which the plasma Ca was increased above the normal level. Elevated values for plasma Ca are seen when toxic amounts of vitamin D are used. However, this procedure also results in an increase in the absorption of Ca, which was undesirable for reasons given previously. It is well known that the plasma level of Ca in the normal rat is relatively unaffected by injections of parathyroid extracts, even when these are given in large doses.

Experiments

A. Three other experiments were conducted with vitamin D free rats in addition to the experiments already described (tables 7 and 9). The diets contained 0.25 per cent Ca. The rats were 24-month-old females except in one experiment, where the animals were 20 months old.

B. Three different experiments were carried out with vitamin D supplemented rats which were given a weekly dose of 70 I.U. vitamin D_2 for at least three weeks before they were taken into the experiments. The rats were 4, 18, and 19 months old; the youngest group consisted of male rats, the other two groups were females.

C. Three experiments were carried out with parathyroidectomized rats. One experiment was performed with vitamin D free rats and two experiments with rats given 70 I.U. vitamin D weekly. The vitamin D free rats were 17 months old, the vitamin D supplemented rats were 6 and 17 months old. Tetany was seen in some of the animals during the first days after the parathyroidectomy and some died. The mortality in the experimental periods was reduced by postponing the experiments for some days in order to let the rats recover from the operation. The serum Ca values of the operated rats were always checked, and those which failed to show a marked reduction in their serum Ca values were discarded.

D. The experiments with thyreotoxic rats were actually carried out for the purpose of determining the correlation of the amount of food eaten to the DJ-Ca. For details see page 49. Serum Ca was reduced in all thyreotoxic rats and all figures have been used for the calculation of the average in table 10.

Table 10. The relationship of digestive juices Ca to plasma Ca

Group	No. of experiments	No. of rats	No. of observations	Serum Ca mg/100 ml	Digestive juices Ca mg/day
Normal, with vitamin D	3	18	35	9.9±0.1	13.6±0.8
Normal, without vitamin D	5	24	76	7.8±0.2	10.3±0.3
Thyreotoxic, without vitamin D	2	12	37	6.0±0.3	7.2±0.4
Parathyroidectomized, with vitamin D	2	10	17	5.2±0.2	5.3±0.3
Parathyroidectomized, without vitamin D	1	5	16	5.0±0.1	5.3±0.2

The figures are averages ± standard error of the mean.

The vitamin D supplemented rats received 70 I.U. vitamin D per week. The diets contained 0.058 per cent or 0.25 per cent Ca with Ca^{45} added; the phosphorus content was 0.4 per cent. The thyreotoxic rats received 0.5 mg sodium L-thyroxin per 10 g diet.

The experimental periods lasted four days, and each animal was usually studied over several periods. Adult rats were used in these studies, of ages ranging from 6 months to two years. The serum Ca values were obtained at the conclusion of each experiment.

Results

The results from these experiments are given in table 10. The DJ-Ca decreases as the concentration of Ca in the blood plasma is lowered, but not in a direct proportion. McLEAN and HASTINGS (1935) published a nomograph from which the concentration of the ionized Ca in the plasma may be determined when the total Ca concentration is known. The plasma proteins were assumed to be constant and 6 g per 100 ml plasma. Although the actual concentration of diffusible Ca will be slightly higher than the concentration of ionized Ca because of the complexing of Ca with organic acids, e.g. citric acid, the calculated ionized Ca will serve well to demonstrate the relationship to DJ-Ca. The plasma concentration of ionized Ca is plotted against the DJ-Ca in figure 5, and the relationship is linear.

The figures given in table 10 for serum Ca are the figures which have been obtained at the end of the experiments. These figures were generally found to be in agreement with those obtained at the start of the experiments; an exception was the fall noted in

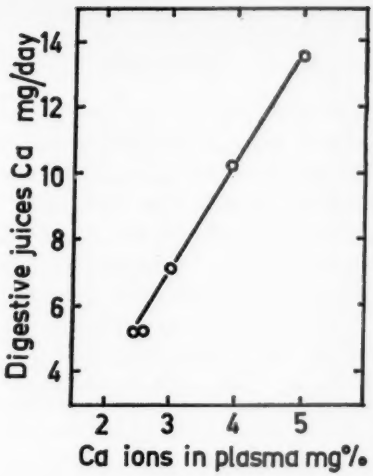

Figure 5

The concentration of ionized Ca in the plasma of rats and the secretion of Ca with the digestive juices.

serum Ca in table 7. It is not known if serum Ca varied from one period to another in a given experiment with vitamin D free rats. The experience from other experiments was that the taking of samples of blood from the tail at too frequent intervals, resulted in a reduction of the dietary intake in the following period in some rats. In the rats which were given vitamin D, plasma Ca, which is well known to be constant, was not checked at the start of the experiments.

A few other observations also need some comments. In contrast to the rats without vitamin D (figure 4), the specific activity of Ca in the urine of rats given vitamin D became constant after two days on the diet.

In the parathyroidectomized rats the effect of vitamin D on the Ca homeostasis was found to be almost negligible.

The reduction of serum Ca in the rats which received thyroxin is marked. It may be possible that this reduction in serum Ca is the result of an increased urinary excretion of citric acid together with Ca. GRAN and STEENBOCK found in unpublished experiments that the citrate was elevated in serum and urine in vitamin D free and vitamin D supplemented rats given thyroxin.

Chapter 10

The amount of food eaten and DJ-Ca

The variations in the concentrations of Ca with the rate of flow of the digestive juices have been discussed previously on page 14 and the following pages. The stimulation of the flow of the digestive juices which follows the intake of a meal may well result in a lowering of the Ca concentration of the digestive juices. Such an effect has been observed, for example, in saliva from rats (DREISBACH 1959) or from humans (BECKS and WAINWRIGHT 1942, 1943); however, the net effect to be expected is an increase in the amount of DJ-Ca. Obviously it is of interest to observe the DJ-Ca as influenced by variations in the daily consumption of diet.

A. In vitamin D supplemented rats

The experimental material obtained with vitamin D supplemented rats (35 observations in 18 rats) was arranged according to the voluntary food intake in the various experimental periods. The rats differed widely in the food intake from one period to another; a low food intake in one period was usually compensated for by a high food intake in the following period. The actual dietary intake in a given period was determined by analyses of the faecal content of chromic oxide. These figures were found to accord with the directly measured food intake; complications arising from constipation or variations in the content of coecum are therefore excluded.

The results are presented in table 11. It appears that an increase in the DJ-Ca follows upon an increase in the food intake. When the DJ-Ca was plotted against the individual figures for the daily food intake in figure 6, the equation for the regression line calculated by the method of the least squares was found to be:

$$y = 0.943\ x + 3.29$$

The coefficient of correlation r was 0.709 ($p < 0.001$). The slope of this line is almost equal to one which would indicate a direct proportionality between the amount of diet eaten and the DJ-Ca. Extrapolation to zero dietary intake gives a value of 3.3 mg DJ-Ca per day, an observation that is in line with the fact that digestive

Table 11. Digestive juices Ca and dietary intake in vitamin D supplemented rats

Range of dietary intake g/day	No. of observations	Average dietary intake g/day	Serum Ca mg/100 ml	Digestive juices Ca mg/day
— 7.0	7	5.5	9.9±0.1	7.6±1.1
7.1—10.0	4	8.9	10.2±0.1	13.2±0.8
10.1—12.0	10	11.2	9.7±0.1	15.2±1.0
12.1—14.0	7	13.2	10.0±0.1	14.1±2.1
14.1 and above	7	16.3	9.9±0.1	18.5±2.2
Mean for 35 observations:		11.2	9.9±0.1	13.6±0.8

The table has been compiled from three experiments on 18 vitamin D supplemented rats. The diets contained 0.25 per cent Ca and 0.4 per cent P, with Ca^{45} added to give a specific activity ranging from 600 to 1,700 cpm/mg Ca.

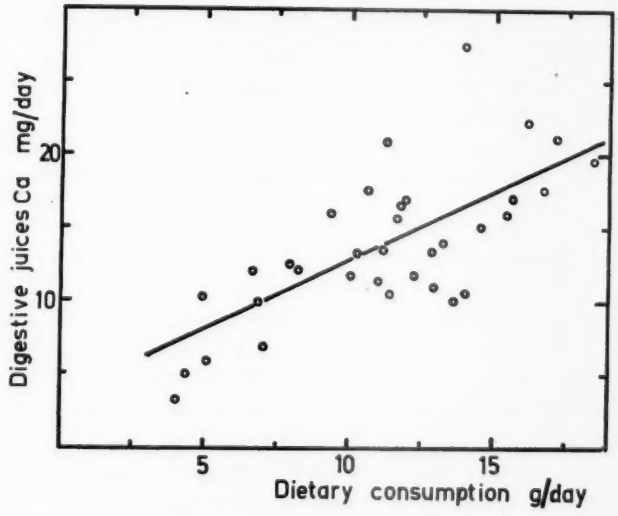

Figure 6
The secretion of Ca with the digestive juices correlated to the daily food intake in vitamin D supplemented rats.

juices are also secreted in the fasting state, although at a much lower rate than when the secretions are adequately stimulated by the voluntary food consumption.

B. In vitamin D free rats

The experimental material obtained with vitamin D free rats (76 observations in 24 rats) was grouped according to the food intake, in the same manner as described for the vitamin D supplemented rats in the preceding section. The figures are presented in table 12. It appears that whereas the DJ-Ca is reduced when the rats consume less than 9–10 g of food daily, the figures appear to be at level when the voluntary food intake is above 10 g daily. Serum Ca was relatively constant. The equation for the regression line was:

$$y = 0.386\ x + 6.16$$

The coefficient of correlation was 0.497 ($p < 0.001$).

It appears that the vitamin D free rats did not increase their DJ-Ca in a direct proportion to the increase in dietary consumption, as did the normal rats.

Table 12. Dietary intake and digestive juices Ca in vitamin D free rats

Range of dietary intake g/day	No. of observations	Average dietary intake g/day	Serum Ca mg/100 ml	Digestive juices Ca mg/day
— 7.0	11	6.1	7.9±0.1	8.0±0.4
7.1— 8.0	3	7.6	7.4±0.3	8.8±1.4
8.1— 9.0	7	8.5	7.5±0.1	10.0±0.8
9.1—10.0	8	9.6	6.7±0.1	10.3±0.7
10.1—11.0	17	10.5	7.9±0.2	10.5±0.6
11.1—12.0	6	11.6	7.4±0.2	11.9±1.1
12.1—13.0	6	12.4	8.1±0.2	11.2±2.1
13.1—14.0	3	13.4	8.0±0.3	11.9±0.5
14.1—15.0	7	14.6	8.4±0.1	10.9±1.5
15.1 and above	8	16.0	7.9±0.3	11.1±1.0
Mean for 76 observations		10.7±0.3	7.8±0.2	10.3±0.3

The table has been compiled from five different experiments on 24 rats. The rats were free of vitamin D, and were from 20 to 24 months old. The diets contained 0.25 per cent Ca and 0.4 per cent P, except in one experiment, where the dietary level of Ca was 0.058 per cent; 0.5 per cent ammonium oxalate was added to this diet, in which the P level was 0.4 per cent.

Additional experimental work was performed in order to discover if the DJ-Ca could be increased above the levels normally found in vitamin D free rats.

a. Two experiments were carried out in which the rats were given the experimental diet with the addition of 0.5 mg sodium L-thyroxin per 10 g of diet. The level of B vitamins was doubled in order to secure sufficient supplies of these vitamins. The rats were vitamin D free females, 14 and 22 months old respectively. The administration of thyroxin was started 12 and 8 days in advance and was continued during the experiments. The food intake of most of these rats, as well as the oxygen consumption, measured in a closed system in five of the rats, was approximately 50 per cent above the initial normal values. Some of these rats, however, did not eat in proportion to the increased energy output and lost weight very severely. Since the purpose was to attain a proper increase in food intake, twelve of the observations which did not fulfil this condition were excluded.
b. In another experiment the volume of the daily food intake was increased by adding 50 per cent cellulose to the diet in a group of four vitamin D free female rats about 1 year old. The expected increase in daily food intake followed.
c. Finally, one experiment was performed with a group of rats on a restricted dietary intake. The food supply was reduced in these rats to 7 g daily. Twenty-four-month old vitamin D free females were used.

The results are given in table 13. It is seen that the DJ-Ca was reduced in all three instances. No correlation to the food intake can be found in these experiments with the exception of the reduction in the DJ-Ca following a restricted food intake. When these rats were later given food *ad libitum* an increase in the DJ-Ca up to the level of the controls was observed. The serum Ca was low in all these rats. However, the relatively large variations in serum Ca may obscure correlations which in reality exist.

When the figures for the groups treated with thyroxin are arranged according to increasing dietay intake in table 14, it appears that the DJ-Ca increased with the food consumption in these rats also. The calculated regression line was:

$$y = 0.35\,x + 2.72$$

and the coefficient of correlation r was 0.289 ($p < 0.2$).

Table 13. Dietary intake and digestive juices Ca in vitamin D free rats

Group	No. of observations	Daily food intake g	Serum Ca mg/100 ml	Digestive juices Ca mg/day
Controls	76	10.7 ±0.3	7.8 ±0.2	10.3 ±0.3
Restricted feeding	5	6.4 ±0.7	8.2 ±0.4	7.8 ±0.9
Thyreotoxic	24	15.4 ±0.5	6.2 ±0.4	8.1 ±0.4
Low caloric diet	4	23.1 ±2.0	6.9 ±0.3	8.5 ±1.4

The figures are means ± standard errors of the mean.

The rats were all vitamin D free animals of ages varying between 14 and 24 months. The control group is the material already presented in tables 10 and 12. Five of these rats were on a restricted dietary intake in one period (7g/day). The thyreotoxic rats were given 0.5 mg sodium L-thyroxin in 10 g diet and the level of B-vitamins was doubled. The administration of thyroxin was started eight or twelve days in advance of the experiment, see table 14. The low caloric diet contained 50 per cent cellulose. The dietary levels of Ca and P were 0.25 per cent and 0.4 per cent respectively.

Table 14. Dietary intake and digestive juices Ca in thyroxin-fed, vitamin D free rats

Range of dietary intake g/day	No. of observations	Average dietary intake g/day	Serum Ca mg/100 ml	Digestive juices Ca mg/day
12.0—14.0	6	12.8	5.5 ±0.2	7.4 ±0.6
14.1—16.0	8	14.7	6.5 ±0.4	8.1 ±0.9
16.1—18.0	7	16.6	6.3 ±0.4	8.1 ±0.7
18.1—20.0	3	19.5	6.4 ±0.7	9.7 ±0.6
Average for 24 observations		15.4	6.2 ±0.4	8.1 ±0.4

Two different experiments were carried out with six female rats deprived of vitamin D in each experiment. The rats in one experiment were 14 months old, and in the other 22 months old. 0.5 mg sodium L-thyroxin was given per 10 g diet, and was started eight and twelve days, respectively, in advance of the actual experiments. The diets contained 0.25 per cent Ca and 0.4 per cent P; the level of B-vitamins was doubled. Ca^{45} was added to the diet.

C. Discussion

An increase in the DJ-Ca proportional to the increase in voluntary food intake was observed in the rats which received an adequate supplement of vitamin D. In the vitamin D free rats, however, the DJ-Ca was found to increase at a much lower rate when the food intake was raised. The difference between the two groups may be due to a failure to increase the rate of secretion of the digestive juices, or to inability to maintain the Ca concentration in the juices following increased rate of secretion, or to a combination of the two suggested factors. More experimental evidence is needed to clear up this problem.

It is most unlikely that the intercept of the extrapolated regression lines with the ordinate at a much higher level in the vitamin D free rats represents a biological reality; and this observation warns against too much reliance on the value of the slope of the line in the vitamin D free rats.

Chapter 11

General discussion

The errors in the analytical methods are discussed on pages 23, 24 and 26.

Some comments are needed on errors due to deviations in the calculated X values (p. 30, Eq. 8). As discussed previously, the counting errors were usually less than two per cent. The value for X in Eq. 8, p. 30 is the calculated result of three different countings. The sum of errors can thus exceed two per cent, but will not be above six per cent. The error in the calculated DJ-Ca depends on the value of X in Eq. 5, p. 29 (see note below).

Ingested Ca: 30 mg daily,
Assumption: DJ-Ca 15 mg daily

a) $X = 0.30$

$Ca_i = 30 \qquad Ca_f = 31.5$

$$Ca_s = \frac{31.5}{1 - 0.3 \pm 0.012} - 30$$

Range for DJ-Ca: 14.2-15.8 mg daily

b) $X = 0.70$

$Ca_i = 30 \qquad Ca_f = 13.5$

$$Ca_s = \frac{13.5}{1 - 0.7 \pm 0.028} - 30$$

Range for DJ-Ca: 11.2-19.6 mg daily.

A 4 per cent error in the estimate for X thus resulted in 5 per cent error in the calculated figure for DJ-Ca when X was 0.3, and nearly 31 per cent when X was 0.7.

The applicability of the method depends upon several assumptions :

1) Complete mixing of ingested Ca with secreted Ca. There can be little doubt about appropriate mixing of saliva and gastric juice into the chyme of the stomach. Some doubt may arise about the events in the duodenum with regard to bile and pancreatic juice, and it is readily conceivable that the intestinal juice will not be properly mixed with ingested Ca.
2) Equal rates of absorption. It follows that this will not occur when incomplete mixing occurs. In fact some of the observations on the absorption of Sr^{90}/Ca^{40} (tables 16 and 19) from 0.25 per cent Ca versus 0.71 per cent Ca diets are readily explained only on the assumption of complete absorption in the jejunal part and a Ca secretion distally.

The observations of LINDQUIST (1952) indicate that the absorption of Ca is completed in the proximal part of the intestine. The observations of HARRISON and HARRISON (1952), however, indicate that additional absorption can take place in the distal part of the small intestine. SCHACHTER and ROSEN (1959) have shown by *in vitro* methods that Ca is mainly absorbed from segments of the upper part of the small intestine. According to NICOLAYSEN's (1951) isolated loop experiments, Ca which is not absorbed is precipitated.

The specific activity of Ca in the upper part of the intestine will be higher than the specific activity of Ca in the lower part of the intestine because of the dilution of the activity with inactive Ca from the digestive juices (MOORE and TYLER 1955). This dilution will be relatively greater when low Ca diets are used. The actual absorption of Ca may therefore be somewhat higher than indicated by the figures for isotope absorption. This possible error is reduced when the absorption is kept low.

Ion exchange between the tissues of the intestinal walls and the intestinal content may also account for isotope disappearance from the lumen, although the mass of Ca may remain the same on both sides of the intestinal epithelium. When the Ca concentration in the lumen is relatively low, relatively more Ca^{45} will be taken up

by the tissues by such mechanisms. Any appreciable error due to ion exchange is ruled out by the results presented in table 7 and figure 4. Isotope uptake from the intestine by ion exchange should decrease with increasing specific activity of plasma Ca. In rats given vitamin D (table 10) the serum specific activity (= urine specific activity) was constant after two days on the labelled diet. The specific activity in serum was about one-half of that in the faeces. Furthermore, the results in table 9, with two different levels of Ca in the diet, seem to exclude the possibility of detectable errors due to ion exchange, since the mass of Ca in the intestinal lumen differed largely.

Secretion of digestive juices with Ca^{45} from plasma are corrected for in the calculation of the absorption coefficient X (Eq. 8, 30). The pasage from jejunum to the faeces, however, introduces a time lag between collections of urine and the faeces representing the DJ-Ca secreted simultaneously with the urine. Observations in man indicate marked individual differences in the rate of transport of food residues through the bowel (ALVAREZ and FREEDLANDER 1924). A correction for this error has not been used in the experiments here presented. It appears from figure 4 that if the urinary collections precede the faecal collections by one to two days, only small changes are introduced in the calculated results for DJ-Ca, since a time lag of two days results in an error in X of less than one per cent. Time lag will of course not cause any error in the vitamin D supplemented rats in which the activity in serum becomes constant after two days of feeding of Ca^{45}.

It appears that although this method is useful when the absorption of Ca is low, serious errors occur when the absorption coefficient X is high. However, X can be reduced when high Ca diets are given to animals with high speed of absorption, and the method will again be reliable, high precision Ca analyses and careful chromic oxide balance technique being prerequisites. Such conditions are fulfilled without serious difficulties. On the other hand, the variations in faecal Ca^{45} will be reduced when the Ca in the diet is too high in comparison with the rate of Ca absorption, and significant changes may therefore be overlooked. Dietary levels above 0.25 per cent Ca were not used in the present work where the speed of Ca absorption was low.

The experiments which were conducted with a low and a moder-

ate level of Ca in the diets (table 9) indicate that the secretion of Ca with the digestive juices is independent of the Ca content of the lumen.

The poorly founded postulate of an active secretion of Ca into the intestine, e.g. colon, in vitamin D deficiency, has been disproved by NICOLAYSEN (1934). NICOLAYSEN and EEG-LARSEN (1953) and NICOLAYSEN *et al.* (1953) discuss the evidence against this postulated effect of the intestine acting as a second kidney. The lower values for DJ-Ca found with vitamin D free rats compared with the DJ-Ca in vitamin D supplemented rats, clearly show that there is no increased intestinal secretion of Ca in vitamin D deficiency.

In one experiment conducted over 32 days (table 7) with vitamin D deficient rats given a low Ca diet with oxalate, the DJ-Ca remained unchanged in spite of the progressive loss of Ca from the body. The organism maintains the concentration of Ca in plasma at an approximately constant level. The actual level, however, depends upon vitamin D and the parathyroids. In view of the finding that the amount of DJ-Ca depends upon the level of Ca in the plasma, it seems improbable that the degree of Ca saturation in the skeleton should affect the DJ-Ca so long as the plasma Ca remains unchanged.

The relationships of DJ-Ca to the level of Ca in the plasma, as well as to the amount of food eaten, were to be expected in view of the experiments quoted in the introduction. The failure of the vitamin D deficient rats to increase the DJ-Ca in the same proportion as the vitamin D supplemented rats when the food intake was increased has already been discussed.

Finally, it may be of interest to observe that HAAVALDSEN, MORTENSEN EGNUND, and NICOLAYSEN (1956) found in old, vitamin D free rats a daily loss of 6 to 12 mg endogenous Ca in the faeces when a nearly Ca free diet with 0.5 per cent sodium oxalate was given. These figures are in the range of the values (4.1—18.3 mg, mean 10.3 mg DJ-Ca daily) found here for vitamin D free rats. In rats supplemented with vitamin D, HAAVALDSEN *et al.* (1956) found a daily loss of 7 to 15 mg Ca when the low Ca —0.5 per cent sodium oxalate diet was given. These values are comparable to the range of 6.4 to 27.6 mg, mean 13.6 mg DJ-Ca found daily in the present experiments.

Part II

LONG TERM EXPERIMENTS ON STRONTIUM-90 METABOLISM

Chapter 12

Introduction

The radioactive fall-out from the nuclear explosions which have been conducted during recent years has resulted in a world-wide contamination by radio-strontium of our foodstuffs and our skeletons (KULP, SCHULERT, and HODGES 1959). Sr^{90} is of special importance because of its long half-life. The atmospheric aspects of Sr^{90} fall-out have been reviewed by MARTELL (1959). The potential hazards caused by atomic radiation have been examined by a number of national committees (MEDICAL RESEARCH COUNCIL 1956, NATIONAL ACADEMY OF SCIENCES 1956) as well as by the UNITED NATIONS SCIENTIFIC COMMITTEE (1958). The INTERNATIONAL COMMISSION ON RADIOLOGICAL PROTECTION (1954), ENGSTRØM *et al.* (1957), and BJØRNERSTEDT and ENGSTRØM (1959) have discussed the hazard problems connected with internal radiation from Sr^{90} in the bone tissues.

Following a perusal of the pertinent literature, the considerations of primary importance from a physiological point of view seem to be the following:

1) The plasma Sr^{90}/Ca will increase and in the course of a given time it will level off and reach a plateau on a given Sr^{90}/Ca level in the diet. Obviously it is of importance to find the time required from zero level to the establishment of the plateau.
2) It follows that in the course of time the skeleton will reach a Sr^{90}/Ca ratio equal to that of the plasma. It is equally important to find the time required to establish this equilibrium. The time required depends upon the age of the individual.

The experimental plan and work are based on the two points outlined. In the following sections such literature will be reviewed as is relevant to the experiments that are to be presented.

The discrimination against Sr

The qualitative differences in chemical properties between Ca and Sr usually result in a preferential biological utilization of Ca. Quantitatively this difference in metabolic utilization of the two elements is expressed by the ratio (COMAR, WASSERMAN and NOLD 1956):

$$\text{Observed Ratio (sample/precursor)} = \frac{\text{Sr/Ca}_{sample}}{\text{Sr/Ca}_{precursor}}$$

which is sometimes called the discrimination factor. This ratio may be determined by measurements of the ratios: Sr/Ca, Sr^{90}/Ca, or Sr^{89}/Ca^{45}, etc.

Major discriminations have been observed in the following processes of Ca and Sr transfer in the body:

1) Preferential absorption of Ca from the intestine,
2) Preferential excretion of Sr in the urine,
3) Preferential secretion of Ca in the milk,
4) Preferential transfer of Ca across the placental wall.

In addition it is possible that minor discriminations (for example blood plasma/bone) may be of some importance.

COMAR, SCOTT-RUSSELL, and WASSERMAN (1957 a) and the UNITED NATIONS SCIENTIFIC COMMITTEE (1958) give general reviews on discriminative processes. In the latter publication a table is given for the discrimination bone/diet in different species. Great variations are seen in the O. R.'s reported for man, which on the average is 0.3–0.4. However, the extremes in man range from 0.17 (in Japan, UNITED NATIONS SCIENTIFIC COMMITTEE 1958) to 0.78 (COMAR *et al.* 1957 a). Wide variations are also found in the other species; the lowest ratios have generally been established by analyses of stable Sr/Ca in the common diet as related to the ratio in the skeleton.

Factors affecting the absorption and discrimination of Sr

When the work here presented was in its first stages, the literature contained rather conflicting reports with regard to the effect

The term Observed Ratio defined above will be abbreviated in the following sections to O. R. (sample/precursor).

of stable Sr and Ca on the uptake of radiostrontium from the intestine. One would expect that dilution with the stable elements of Ca and Sr, i.e. a reduction of the ratio Sr^{90}/Sr or Sr^{90}/Ca in the intestine, would result in a correspondingly reduced absorption and retention of Sr^{90}. In fact, results in confirmation of such an expectation were reported (WASSERMAN, COMAR and PAPADOPOULOU 1957) at that time. The outcome of the work here presented is a full substantiation of the expectation. A survey of the pertinent literature indicates the confusion that readily arises out of much conflicting evidence.

COPP, AXELROD, and HAMILTON (1947), McDONALD *et al.* (1954, 1955), COPP and KAWIN (1956), WASSERMAN *et al.* (1957), and PALMER, THOMPSON, and KORNBERG (1958), have shown that rats on low Ca diets retained considerably more of ingested radio-Sr than animals with higher levels of Ca in their diets. KIDMAN, TUTT, and VAUGHAN (1950), and JOWSEY *et al.* (1955) found that rabbits on low Ca diets retained more of parenterally administered Sr^{90} than rabbits on a higher Ca intake. Parenteral administrations of relatively small amounts of stable Sr mixed with Sr^{85} or Sr^{90} depressed the skeletal retention of radio-Sr in rats (HAMILTON 1948, CATSCH 1957). ENGSTRÖM *et al.* (1957) refer to CARLQVIST and NELSON, who found that the excretions of Sr^{89} and Sr^{90} were increased after large amounts of administered inactive Sr.

COMAR and WASSERMAN (1957) gave Sr^{89} and Ca^{45} with increasing amounts of stable Ca to rats by stomach tube. The Sr^{89} uptake in the bones was not depressed following the dilution with Ca^{40}, but the Ca^{45} uptake was somewhat reduced. McDONALD *et al.* (1955) found that the Ca content of a single dose given by stomach tube did not affect the absorption of Sr^{90} to the extent expected. GROSS, TAYLOR, and WATSON (1954), and HARRISON, JONES, and SUTTON (1957) found that stable Sr given as carrier together with radio-Sr did not extensively reduce the retention of radio-Sr when given to rats by stomach tube. KIDMAN *et al.* (1950) stated that stable Sr did not seem to have any effect on the retention and excretion of Sr^{89} after intravenous injection in rabbits.

COPP *et al.* (1947) and COPP and KAWIN (1956) found that young rats given radio-Sr orally in a single dose retained more of

the isotope than old animals. COMAR and WASSERMAN (1957) confirm this observation, and found in addition that there was significantly less discrimination in the young rats. On the other hand, lifetime feeding of Sr^{90} showed that there was little difference in the discrimination in rats between 70 and 400 g of body weight (COMAR, WHITNEY, and LENGEMANN 1955).

According to the experiments of WASSERMAN *et al.* (1957) the O. R. (body/diet) remained unchanged, although the retentions of Sr^{89} and Ca^{45} were depressed in proportion to an increase in the dietary Ca from 0.5 to 2.0 per cent. PALMER, THOMPSON, and KORNBERG (1958) varied the Ca content of the diet, phosphate being constant. The O. R. (femur/diet) was increased 2–3 times when Ca in the diet was increased from 0.1 to 2 per cent.

COMAR, WASSERMAN, and NOLD (1956), COMAR *et al.* (1957 b), COMAR and WASSERMAN (1956, 1957), LENGEMANN, COMAR, and WASSERMAN (1957), and LENGEMANN, WASSERMAN, and COMAR (1959), have found that the O. R. (body/diet) is almost doubled when dietary Ca is supplied by milk, or if lactose is added to the diet, compared to other sources of Ca. Such results were obtained in man, cattle, and rat, but not in the rabbit.

The elimination of Sr from the body

Of a given amount of Sr/Ca, relatively more Sr than Ca will be excreted in the urine as well as in the faeces. The relative importance of the two routes of elimination is dependent upon the method by which the isotope is given. Oral intakes result in a higher ratio of faecal to urinary excretion because of the absorptive discrimination, than does parenteral administration. The endogenous Sr in faeces derives from the secretion of Sr with Ca in the digestive juices.

COMAR *et al.* (1957 b) found that approximately six per cent of the daily oral dose was excreted in the urine in adult patients; the excretions were reduced when the patients were transferred to a milk diet, although the absorption was increased. On the other hand, COMAR *et al.* (1956) found that in rats 8.5 per cent of the daily oral Sr^{89} intake was eliminated in the urine when the rats were on a milk diet, in contrast to 1.9 per cent when a com-

mercial diet was fed. Expressed in per cent of the absorbed dose of Sr^{89}; the values were equal and independent of the type of diet.

The urine is the main route of Sr elimination in man after parenteral administration (HARRISON, RAYMOND and TRETHEWAY 1955, McCANCE and WIDDOWSON 1939, and SPENCER, LASZLO and BROTHERS 1957). DURBIN *et al.* (1957) obtained similar results in the rhesus monkey. However, following parenteral administration of radio-strontium on a low Ca diet, the faecal excretion always exceeded the urinary elimination in the rabbit (KIDMAN *et al.* 1950).

PECHER (1941) and LIKINS *et al.* (1959) found in mice and rats, respectively, a higher faecal than urinary isotope excretion following parenteral administration. In contrast, BAUER, CARLSON and LINDQUIST (1956) observed in rats a somewhat higher urinary excretion.

SINGER *et al.* (1957) found that Sr^{89} was secreted into the alimentary canal in preference to Ca.

The fixation of Sr in the bones

The mechanisms for the skeletal fixation of Ca and Sr have not been fully elucidated. However, ion exchange and bone accretion (see footnote) result in the accumulation of Sr. The considerable discriminations in the intestine and in the kidney have made it difficult technically to establish if there is a minor discrimination against Sr in the transfer blood-bone.

Experiments with bone grown *in vitro* (LENGEMANN, 1957) resulted in an O. R. (bone/medium) of 0.83. NEUMAN (1958) found that the rate and the extent of Sr^{90} uptake in a synthetic hydroxyapatite were approximately equal to those found in experiments with Ca^{45}.

The following definitions have been used:
Accretion: Total amount of bone salt deposited in the skeleton.
Resorption: The total amount of bone salt brought into solution.
Retention: The net deposition of bone salt, i. e. the difference between accretion and resorption.
Absorption: The net amount taken up from the digestive tract. This term should not be confused with the *resorption* which is sometimes used in the same sense in the German and Scandinavian literature.

In vivo experiments in man, rats, and goats, indicate that the skeleton does not distinguish between Ca and Sr (BAUER *et al.* 1955 a, COMAR *et al.* 1956, 1957 b, BAUER 1957, COMAR and WASSERMAN 1957, McDONALD, NOYES, and LORICK 1957, TALMAGE, SCHOOLEY, and COMAR 1957, and WASSERMAN, LENGEMANN, and COMAR 1958).

VAN CLEAVE and KAYLOR (1958) found that the bone shaft of femur discriminated less against Sr than the end portions. The ratio of Sr/Ca in serum decreased with time after injection. Female rats weighing between 160 and 200 g were used. LIKINS *et al.* (1959) found that the ratio in serum remained constant with time, whereas there was a fall in the O. R. (bone/plasma). These authors describe this result as due to a preferential release of Sr^{89} from the skeleton. Weanling rats weighing between 40 and 50 g were used in this study.

It is possible that this discrepancy in the results is caused by the differences in size of the rats. The more rapid turnover of the radioisotopes in the processes of ion exchange, bone accretion, and bone resorption in the youngest rats (LIKINS *et al.* 1959) and in the epiphyseal ends of femur (VAN CLEAVE and KAYLOR 1958) may lead to a preferential loss of Sr. The failure of LIKINS *et al.* (1959) to observe a fall in the O. R. in serum may have been accidental and due to a balance in the excretion and in the release of Sr from the skeleton.

The distribution of Sr in the skeleton

A division must be made between a continuous exposure to a contaminated diet and the retention of a single dose of radioactive Sr. In the first instance measurements of stable Sr in human bones (SOWDEN and STITCH 1957, THURBER *et al.* 1958), and of Sr^{90} in bones from rats given a Sr^{90} containing diet over a lifetime (COMAR, WHITNEY, and LENGEMANN 1955), show that Sr is uniformly distributed in the various bones in the skeleton.

KULP, ECKELMANN, and SCHULERT (1957) found that the distribution of Sr^{85} in the human skeleton differed markedly in the various bones after the administration of the isotope in a single dose. WASSERMAN *et al.* (1958) arrived at the same con-

clusion in goats. Numerous other studies confirm this point; the highest amounts of Sr per unit Ca are found in the trabecular bones, i.e. vertebrae and ribs. The radioactivity will concentrate in "hot-spots" both in trabecular and in compact bone.

The skeletal ratio of Sr/Ca

HODGES *et al.* (1950) found that the skeletal ratio of stable Sr/Ca in humans was constant after two years of age, whereas SOWDEN and STITCH (1957) found a tendency to higher values for stable Sr/Ca in the older age groups. In adults, Thurber *et al.* (1958) found no observable differences related to age in a material of 745 cases. The results of ALEXANDER and NUSBAUM (1959) suggest that the ratio Sr/Ca increases until the age of twelve years. The regional and individual variations appear to be great. A serious limitation in the interpretation of the results quoted above is that the ratio Sr/Ca in the diet eaten most probably also increases as the individuals grow older. In rats, where the ratio of Sr^{90}/Ca in the diet remained constant over the lifetime of the rats, COMAR *et al.* (1955) concluded that the ratio of Sr^{90}/Ca in the body remained unchanged throughout life.

Results for Sr^{90} in human bones show that the highest amounts are found in the youngest age groups (KULP, ECKELMANN and SCHULERT 1957, ECKELMANN, KULP and SCHULERT 1958, and KULP, SCHULERT and HODGES 1959). In the last paper the highest ratio of Sr^{90}/Ca was found in bones taken from children in the age group between two and three years. It is not known if this ratio will increase provided the ratio Sr^{90}/Ca in the diet remains constant.

The lower figures for Sr and Sr^{90} found in the skeletons of very young children are explained by the fact that much of their body Ca is derived from the mothers during gestation and lactation. In addition, discriminations against Sr have been reported in the transfer of Ca and Sr across the placental wall (COMAR *et al.* 1955) and in the secretion of milk.

Chapter 13

Plan of experiments

It is well established that the body discriminates against Sr in favour of Ca. Major discriminations against Sr take place in the intestine and in the kidneys. It appears, however, that the discrimination against Sr varies considerably. In addition to the discriminations against Sr, the available evidence indicates that the age of the body, e.g. the state of growth and Ca retention, as well as the levels of Sr and Ca in the diet, are very important factors in the retention of Sr^{90}. The observed ratios for the individual processes have been determined mostly by short-term experiments, and a full prediction of the following pertinent points is therefore difficult: a) the final Sr^{90}/Ca level in the skeleton, and b) the time required to establish this level. Both points may be a function of age.

The investigations presented below were therefore started in order to elucidate the problems outlined above. Long-term experiments on diets with a constant level of Sr^{90}/Ca were a prerequisite. The actual situation in human nutrition today with a varying and increasing contamination with Sr^{90} could not be imitated because of great difficulties in design, operation, and interpretation of the experiments.

The work was planned in order to investigate the following questions:

1) The absorption, excretion, and retention of Ca and Sr^{90} as related to age.
2) The absorption, excretion, and retention of Sr^{90} as related to the level of Ca intake.
3) The times required before the establishment of a constant proportion of Sr^{90}/Ca in the plasma and in the skeleton, respectively.
4) The length of time required before Sr^{90} balance is established.

The experiments were carried out by long-term balances divided in metabolic periods of four to seven days duration. The diets were planned to contain approximately 0.25 per cent Ca, which is known to be just sufficient to cover the requirement in growth and development in the rat (SHERMAN 1947). It was thought

that this dietary level of Ca might give a relatively representative picture of the situation in human nutrition in many countries with respect to the Ca intake, e.g. the Far East and South Africa (cf. UNITED NATIONS SCIENTIFIC COMMITTEE 1958). One experiment was carried out with a higher level of dietary Ca, 0.71 per cent. In order to provide sufficient phosphorus for tissue syntheses, the diets contained 0.4 per cent P as primary potassium phosphate.

Sr^{90} was used in these investigations because the interpretations of the results were facilitated when the radioactive decay did not have to be considered. The interpretation of the results would thus have been very difficult if Sr^{89} had been used in the long-term experiments. Ca^{45} was not used in the long-term experiments for the same reasons.

Chapter 14

Experimental methods

The experimental methods were to a large extent identical with those described in chapter 3; part I.

Rats: Young rats were taken from the colony of the Institute. The animals were given the pre-experimental diet previously described (table 2) from the time of weaning until they were taken into experiments. A weekly supplement of 70 I.U. vitamin D_2 was given in addition to vitamin A as described.

Diets: The diets are described in table 2. The composition of the experimental diet was changed somewhat from the diet used previously in part I by adding ten per cent hardened coconut fat. The purpose was to make the diet sticky and non-dust-producing in order to prevent spreading of the radioactivity to the surroundings. The level of Ca was varied, as will be described below.

It was desirable to know the content of stable Sr in the diets. The Sr content was determined by emission spectrography. The pre-experimental diet contained 1690 micrograms Sr per g Ca, the experimental diets approximately 700 micrograms Sr per g Ca. The lower content found in the latter diet is most probably due to the replacement of ground whole wheat in the pre-experimental diet by wheat flour of 70 per cent extraction in the experimental diet.

Balance experiments: The balance studies were conducted as described on page 23, with 0.5 per cent chromic oxide added to the diets. The experimental periods lasted from four to seven days; the length of each period is indicates in the tables.

Analytical: The analytical techniques have been described on pp. 24 *et seq.*

EXPERIMENTAL RESULTS

The results are described in the following order: long-term balance studies, the ratio of Sr^{90}/Ca in the body, the ratio of Sr^{90}/Ca in the serum, Observed Ratios, elimination of Sr^{90}, double tracer experiments, and the absorption of Ca from a non-milk versus a milk diet.

Chapter 15

Long term balance studies

The retention and accumulation of Sr^{90} was studied in consecutive metabolic periods; Sr^{90} was mixed into the diets.

The following experiments were conducted with a level of 0.25 per cent Ca and 0.4 per cent P in the diet:

a) Six male rats, 28 days old at start, were continued in the experiment for 50 days (table 15).
b) Six male rats, 70 days old at start, were continued in the experiment for 103 days (table 16).
c) Six male rats, 100 days old at start, were continued in the experiment for 62 days (table 17).
d) Six male rats, 156 days old at start, were continued in the experiment for 53 days (table 18).

The level of Sr^{90} varied in the different experiments, but remained constant in each group. The figures for the Sr^{90} content are given in the tables.

Next, one experiment was carried out with a dietary level of 0.71 per cent Ca and 0.5 per cent P:

Six rats of both sexes, 66 days old at the time of start, were studied on this diet for 59 days (table 19).

Results

The results from the long-term balance studies are given in tables 15–19. The length of each metabolic period is indicated.

The absorption of Ca and Sr^{90}

Since the level of Ca in the diet was constant, the daily Ca intake in the different groups increased in proportion to the rate of growth. In the first experimental period the Ca intake is relatively low. However, these periods have to be considered as periods of adaptation of the rats to new environments and a new type of diet.

In the youngest rats (table 15) the net Ca absorption remained high (about 80 per cent) throughout the experiment. In the other experiments the net absorption declined as expected (HAAVALDSEN and NICOLAYSEN 1956). The rats on the 0.71 per cent Ca diet (table 19), did not absorb more Ca than rats of comparable age on the 0.25 per cent Ca diets. The implication is that the 0.25 per cent level covered the requirement.

Sr^{90} in the faeces, calculated as per cent of the intake, shows two characteristic features. Without exception in all five experiments, Sr^{90} in faeces increases in the course of the experiment. It is definitely correlated to the age of the animal, meaning a correlation to the efficiency of the absorption of Ca.

The effect of an increased level of Ca in the diet on the Sr^{90} absorption is striking. A comparison of tables 19 and 16 is instructive in this respect.

The O. R. (absorption) will later be discussed separately on page 77.

Urinary excretion

The urinary level of Ca was low in all the experiments. In some experiments a tendency to increase occurred, in others the levels remained constant. Such low levels of Ca in the urine and also irregular variability are well known from other experiments. In HAAVALDSEN and NICOLAYSEN's (1956) experiments, the urinary Ca varied irregularly between 0.3 and 1.2 mg daily when the Ca level of the diet was 0.5 per cent.

Sr^{90} in the urine remained within ten per cent of the intake

Table 15. The absorption and retention of Sr^{90} in relation to Ca balance in rats 28 days old on continuous ingestion of Sr^{90}
Experiment No. Sr-12

Period Number	Age days	Body weight g	Ca Intake mg/day	Ca Absorption mg/day	Sr^{90} in faeces % of intake	Ca in urine mg/day	Sr^{90} in urine % of intake	Observed Ratio (absorption) Sr^{90}/Ca Direct	Corrected
1	28	54	16.9 ± 1.0	15.1 ± 1.0	29.5 ± 2.3		4.8 ± 0.4	0.79 ± 0.02	0.75
2	32	67	20.3 ± 1.8	18.6 ± 1.0	33.4 ± 3.5		3.2 ± 0.3	0.73 ± 0.04	0.70
3	36	81	21.9 ± 0.9	19.9 ± 0.8	32.6 ± 2.6	0.1 ± 0.03	3.5 ± 0.5	0.74 ± 0.03	0.71
4	46	114	21.3 ± 2.7	19.4 ± 2.6	30.9 ± 2.5	0.2 ± 0.03	6.9 ± 1.5	0.76 ± 0.02	0.73
5	50	128	24.3 ± 2.0	22.1 ± 1.8	29.9 ± 1.9		11.8 ± 2.3	0.77 ± 0.03	0.74
6	57	147	27.0 ± 2.2	23.7 ± 2.2	39.0 ± 2.4	0.3 ± 0.08	10.1 ± 1.2	0.69 ± 0.02	0.66
7	64	162	27.7 ± 1.3	24.9 ± 1.1	41.6 ± 2.5		10.5 ± 0.9	0.65 ± 0.03	0.63
8	71-78	176	24.6 ± 1.7	21.5 ± 1.7	48.0 ± 4.6	0.3 ± 0.02	6.6 ± 0.8	0.59 ± 0.05	0.56

Mean ± standard error of the mean for six male rats. Following weaning the rats received the stock diet. From their 28th day of life the rats received the radioactive diet with 0.25 per cent Ca and 0.4 per cent P. 15 μC Sr^{90} was added per 5 kg diet in the form of a solution of Sr^{90}-Y^{90} nitrates. 1 mC of this solution contained 0.1 mg solids. The activity was 3,080 cpm per g diet.
The diet contained 700 microg. Sr per g Ca.
The Observed Ratio (absorption) corrected has been corrected for 15 mg DJ-Ca/day. Serum Ca at the end of the experiment was 10.4 ± 0.2 mg/100 ml.

Table 16. The absorption and retention of Sr^{90} in relation to Ca balance in rats 70 days old on continuous ingestion of Sr^{90}
Experiment No. Sr-8

Period	Age	Body weight	Ca intake	Ca	Sr^{90}	Ca	Sr^{90}	Observed Ratio (absorption)

Table 16. The absorption and retention of Sr^{90} in relation to Ca balance in rats 70 days old on continuous ingestion of Sr^{90}
Experiment No. Sr-8

Period number	Age days	Body weight g	Ca intake mg/day	Ca absorption mg/day	Sr^{90} in faeces % of intake	Ca in urine mg/day	Sr^{90} in urine % of intake	Observed Ratio (absorption) Sr^{90}/Ca Direct	Corrected DJ-Ca	Corrected DJ-Ca + DJ-Sr^{90}
1	70	142	19.4 ± 1.5	18.1 ± 1.5	26.4 ± 2.2	0.1 ± 0.0	5.7 ± 1.0	0.79 ± .02	0.77	0.82
2	74	157	31.3 ± 1.4	30.0 ± 1.4	22.3 ± 1.8		6.6 ± 0.2	0.81 ± .02	0.80	0.83
3	78	170	25.7 ± 1.6	24.3 ± 1.4	26.3 ± 1.9		7.3 ± 1.4	0.78 ± .02	0.76	0.81
4	82	182	26.6 ± 1.5	25.2 ± 1.4	25.0 ± 3.1		6.9 ± 1.0	0.79 ± .03	0.78	0.81
5	89	195	27.3 ± 1.5	25.3 ± 1.4	31.5 ± 3.1		9.0 ± 0.6	0.74 ± .03	0.72	0.77
6	96	214	29.2 ± 1.7	26.5 ± 1.6	33.6 ± 3.8	0.2 ± 0.0	10.2 ± 0.4	0.74 ± .04	0.71	0.76
7	103	228	31.2 ± 0.9	25.2 ± 1.0	53.5 ± 3.6		10.2 ± 0.9	0.58 ± .03	0.53	0.62
8	110	235	36.0 ± 1.5	21.3 ± 1.4	77.5 ± 2.3	0.6 ± 0.1	6.4 ± 0.9	0.38 ± .02	0.32	0.45
9	117	247	31.1 ± 1.8	16.9 ± 1.5	78.0 ± 1.8		5.6 ± 0.6	0.41 ± .03	0.32	0.48
10	124	255	35.3 ± 1.1	16.4 ± 1.2	81.9 ± 1.0	0.7 ± 0.1	6.4 ± 0.4	0.39 ± .01	0.29	0.45
11	131	265	35.0 ± 1.0	12.6 ± 0.5	83.4 ± 1.0	0.7 ± 0.1	5.6 ± 0.6	0.46 ± .03	0.30	0.49
12	138	275	33.9 ± 0.8	14.2 ± 0.7	83.4 ± 0.7	0.7 ± 0.1	5.5 ± 0.5	0.40 ± .02	0.28	0.46
13	145	282	35.4 ± 0.9	12.4 ± 0.9	86.0 ± 0.7	1.0 ± 0.1	6.9 ± 0.4	0.40 ± .03	0.26	0.45
14	152	284	36.2 ± 1.4	11.0 ± 0.9	89.0 ± 1.7	1.2 ± 0.1	6.1 ± 0.4	0.37 ± .07	0.22	0.44
15	159	297	35.2 ± 1.4	10.8 ± 0.6	87.8 ± 1.0		6.4 ± 0.3	0.40 ± .02	0.24	0.45
16	166-73	296	37.4 ± 0.9	9.4 ± 0.4	91.6 ± 0.3	1.3 ± 0.1	6.7 ± 0.3	0.33 ± .01	0.18	0.41

The figures are mean ± standard error of the mean for six male rats. Following weaning the rats were fed the pre-experimental diet with 0.25 per cent Ca and 0.4 per cent P. From their 70th day of life the rats received the radioactive diet with 0.25 per cent Ca, 0.4 per cent P, and 6930 cpm Sr^{90} per g diet. 32 μC Sr^{90} was added per 5 kg diet in the form of a solution of Sr^{90}-Y^{90} nitrates 1 mC of this solution contained 0.1 mg solids. The diet contained 700 microg. Sr per g Ca.

The Observed Ratio (absorption) corrected has been corrected for 15 mg DJ-Ca/day or 15 mg DJ-Ca/day and 13,400 cpm DJ-Sr^{90}/day. Serum Ca at the end of the experiment was 11.0 ± 0.1 mg/100 ml, Sr^{90} activity in serum was 894 cpm/mg Ca.

Table 17. The absorption and retention of Sr^{90} in relation to Ca in rats 100 days old on continuous ingestion of Sr^{90}
Experiment No. Sr-9

Period number	Age days	Body weight g	Ca intake mg/day	Ca absorption mg/day	Sr^{90} in faeces % of intake	Sr^{90} in urine % of intake	Observed Ratio (absorption) Sr^{90}/Ca Direct	Corrected
1	100	230	12.7 ±1.6	8.3 ±1.1	66.8 ±1.1	7.4 ±0.9	0.51 ±0.02	0.39
2	102	238	28.5 ±2.4	19.4 ±1.9	67.1 ±2.3	5.6 ±0.6	0.48 ±0.03	0.42
3	106	245	28.7 ±1.8	18.3 ±1.2	67.9 ±1.9	5.8 ±0.2	0.50 ±0.01	0.42
4	113	248	32.0 ±1.3	19.7 ±1.3	67.8 ±1.5	5.6 ±0.3	0.52 ±0.00	0.44
5	120	256	34.6 ±1.7	17.1 ±0.9	74.3 ±1.6	4.1 ±0.6	0.55 ±0.03	0.41
6	127	266	34.3 ±1.5	13.3 ±0.7	82.1 ±1.5	3.5 ±0.5	0.46 ±0.06	0.31
7	134	273	34.8 ±1.6	14.8 ±0.9	86.2 ±0.7	3.4 ±0.8	0.32 ±0.01	0.23
8	141	27[illegible]	34.2 ±1.0	11.1 ±0.3	84.8 ±0.4	3.0 ±0.2	0.48 ±0.01	0.29
9	148	285	37.0 ±0.9	11.4 ±0.9	87.0 ±1.4	5.1 ±0.4	0.42 ±0.03	0.26
10	155-62	286	35.4 ±1.0	9.8 ±0.5	88.8 ±1.4	4.3 ±0.4	0.40 ±0.04	0.23

The figures are mean ± standard error of the mean for six male rats. Following weaning the rats were fed the pre-experimental diet with 0.25 per cent Ca and 0.4 per cent P. From their 100th day of age the rats were given the radioactive diet with 0.25 per cent Ca and 0.4 per cent P. The activity was 6,930 cpm Sr^{90}/g diet. 32 μC Sr^{90} was added per 5 kg diet in the form of a solution of Sr^{90}-Y^{90} nitrates, 1 mC of this solution contained 0.1 mg solids. The diet contained 700 microg. Sr per g Ca.
The Observed Ratio (absorption) corrected has been corrected for 15 mg DJ-Ca/day.
Serum Ca at the conclusion of the experiment was 10.5 ± 0.05 mg/100 ml.
Urinary Ca was almost constant during the experiment, with a daily figure of 0.7 mg/day.

Table 18. The absorption [illegible]

Urinary Ca was almost constant during

Table 18. The absorption and retention of sr^{90} in relation to Ca balance in rats 156 days old on continuous ingestion of Sr^{90}
Experiment No. Sr-10

Period number	Age days	Body weight g	Ca intake mg/day	Ca absorbed mg/day	Sr^{90} in faeces % of intake	Ca in urine mg/day	Sr^{90} in urine % of intake	Observed Ratio (absorption) Sr^{90}/Ca Direct	Corrected
1	156	288	23.4 ±3.1	11.5 ±1.5	80.1 ±2.0		12.5 ±1.4	0.41 ±0.01	0.29
2	160	299	32.0 ±2.6	11.2 ±1.9	87.1 ±4.1	1.9 ±0.3	7.0 ±1.5	0.37 ±0.05	0.23
3	167	310	37.7 ±1.5	14.6 ±2.6	84.9 ±2.8		5.8 ±0.6	0.39 ±0.05	0.29
4	174	303	37.2 ±2.3	10.9 ±1.1	88.4 ±1.9	1.3 ±0.02	6.1 ±0.8	0.40 ±0.04	0.25
5	181	318	31.0 ±1.4	7.1 ±1.1	93.4 ±2.0		5.2 ±0.7	0.29 ±0.03	0.14
6	188	299	35.7 ±2.1	8.2 ±0.3	91.9 ±1.4	1.2 ±0.02	4.7 ±0.5	0.35 ±0.07	0.18
7	195	320	30.8 ±1.9	6.3 ±0.6	95.1 ±1.0		5.0 ±0.5	0.24 ±0.06	0.11
8	202-9	321	36.0 ±2.0	7.8 ±0.8	95.6 ±1.3	1.3 ±0.03	5.3 ±0.5	0.20 ±0.04	0.10

The figures are means ± standard error of the mean for six male rats. Following weaning the rats were fed the pre-experimental diet with 0.25 per cent Ca and 0.4 per cent P. From their 156th day of life the rats were given the radioactive diet with 0.25 per cent Ca and 0.4 per cent P. The activity was 3,080 cpm Sr^{09}/ g diet. 15μC Sr^{90} was added per 5 kg diet in the form of a solution of Sr^{90}-Y^{90} nitrates, 1mC of this solution contained 0.1 mg solids. The diet contained 700 microg. Sr per g Ca.
The Observed Ratio (absorption) corrected has been corrected for 15 mg DJ-Ca /day.
Serum Ca at the end of the experiment was 10.0 ± 0.2 mg /100 ml.

Table 19. The absorption and retention of Sr^{90} in relation to Ca balance in rats 66 days old on continuous ingestion of Sr^{90} with high Ca in their diet
Experiment No. Sr-11

Period number	Age days	Body weight g	Ca intake mg/day	Ca absorption mg/day	Sr^{90} in faeces % of intake	Ca in urine mg/day	Sr^{90} in urine % of intake	Observed Ratio (absorption) Sr^{90}/Ca Direct	Corrected
1	66	163	71.1 ±8.0	27.3 ±2.9	86.6 ±5.3		2.0 ±0.1	0.35 ±0.08	0.27
2	68	169	82.6 ±4.1	29.1 ±1.8	84.2 ±3.3	0.5 ±0.02	1.2 ±0.1	0.45 ±0.08	0.35
3	72	171	77.7 ±2.8	25.0 ±1.3	84.9 ±3.7		1.2 ±0.0	0.47 ±0.08	0.35
4	76	183	87.4 ±3.5	23.7 ±1.7	85.7 ±2.5	1.1 ±0.02	2.1 ±0.0	0.53 ±0.08	0.38
5	83	190	86.1 ±3.4	20.1 ±1.4	90.4 ±2.7		2.1 ±0.0	0.41 ±0.10	0.28
6	90	196	86.9 ±2.7	16.0 ±1.0	88.6 ±1.4	0.4 ±0.02	1.5 ±0.1	0.62 ±0.05	0.38
7	100	203	85.5 ±1.0	15.9 ±1.0	97.3 ±1.6		3.1 ±0.1	0.15 ±0.07	0.09
8	104	201	75.9 ±3.5	10.6 ±1.5	97.7 ±2.3	0.7 ±0.04	1.9 ±0.1	0.16 ±0.08	0.08
9	111	206	93.0 ±4.4	10.3 ±1.2	99.3 ±2.1		2.0 ±0.1	0.06 ±0.03	0.03
10	118-25	218	80.0 ±3.6	11.0 ±0.8	94.5 ±2.0	0.5 ±0.07	2.5 ±0.1	0.40 ±0.10	0.20

The figures are means ± standard error of the mean for six rats, three of each sex. Following weaning, the rats were fed the pre-experimental diet with 0.25 per cent Ca and 0.4 per cent P. From their 65th day of life, the rats were given the radioactive diet with 0.71 per cent Ca and 0.5 per cent P. Balances were started one day later. The activity was 2.990 cpm Sr^{90} per g diet. 15 μC Sr^{90} was added per 5 kg diet in the form of a solution of Sr^{90}-Y^{90} nitrates, 1 mC of this solution contained 0.1 mg solids. The Observed Ratio (absorption) corrected has been corrected for 15 mg DJ-Ca/day. Serum Ca at the end of the experiment was 12.5 ± 0.05 mg/100 ml.

in practically all observations. Evidently there is a correlation between Sr^{90} absorbed and the urinary output of Sr^{90} when the different groups are compared. It is quite interesting to observe that, in spite of some irregularities, a certain level is established even from the early periods. This reflects the establishment of a very early constant level in the plasma, as discussed later on page 76.

Retention

The consequence of an output of Sr^{90} in faeces which increases with time, and of the constant level in the urine, is a decreasing retention of Sr^{90}.

Chapter 16

The ratio of Sr^{90}/Ca

A. The ratio of Sr^{90}/Ca in the body

The total body Ca in the rats reported on in table 16 was taken to be 0.70 per cent of the body weight at the start of this experiment. This assumption is based on the figures given by SHERMAN (1947) for the Ca content of rats of the same age fed on 0.25 per cent Ca and 0.4 per cent P from weaning, i.e. the levels of our pre-experimental diet. The increments in the body Ca follow from the balances in these rats. Consequently, Sr^{90}/Ca in the body can be calculated, and these figures are given in table 20. It is seen that the rats continued to retain Sr^{90} until the end of the experiment. However, the ratio of Sr^{90}/Ca in the body reached a maximum value after 33 days on the radioactive diet (period 7, table 16). A slight fall next occurs. The absorption of Sr^{90} per mg Ca was constant in the periods to follow.

At the end of this experiment, by analyses the average ratio of Sr^{90}/Ca in the serum was found to be 894 cpm/mg Ca; thus the agreement with the calculated figure (836 cpm/mg Ca) for Sr^{90}/Ca of the whole body is remarkable.

Identical calculations were carried out for the other four experiments. The initial values for the skeletal Ca content were taken from table 20 (Exp. No. Sr-8) with the exception of the experiment in table 15 (Exp. Sr-12) where the figures of SHER-

Table 20. Calculated activity for Sr^{90} per mg Ca in the body
Experiment Sr-8

Period number	Body weight at the start of the period g	Calcium in the body at end of the period mg	Sr^{90} per mg calcium in body at the end of the period cpm/mg	Sr^{90} per mg calcium absorbed during the period cpm/mg
1	142	1066	136	2180
2	157	1185	330	2260
3	170	1282	449	2157
4	182	1458	637	2200
5	195	1634	759	2051
6	214	1818	859	2046
7	228	1993	890	1549
8	235	2138	880	1042
9	247	2252	878	1122
10	255	2362	870	1077
11	265	2446	869	1287
12	275	2541	864	1105
13	282	2621	856	1156
14	284	2690	847	1098
15	297	2757	840	1199
16	296	2814	836	1026

The Sr^{90} level per mg Ca was 2,760 cpm in the diet.

The data are from the same rats as were reported on in table 16. The Ca content of the body was assumed to be 995 mg at the start of the experiment, using the data of SHERMAN (1947) and previous experience from this laboratory (0.70 % Ca).
At the conclusion of the experiment, the strontium - 90 activity in serum was 894 cpm per mg Ca. This value was established by direct analyses on the pooled sample of blood serum from the rats.

MAN (1947) were used. In order to present the different experiments in the same figure, the O.R.'s (body/diet) have been calculated according to the equation given on page 58. The O. R.'s (body/diet) are plotted against the age of the rats in figure 7. The different experiments are indicated by references to the experiment numbers and the corresponding tables.

The experiments given in table 16 (Exp. Sr-8) are first discussed. A peak is observed at the age of 103 to 110 days, which corresponds to the 7th—8th period, whereafter a slight decline was observed. In

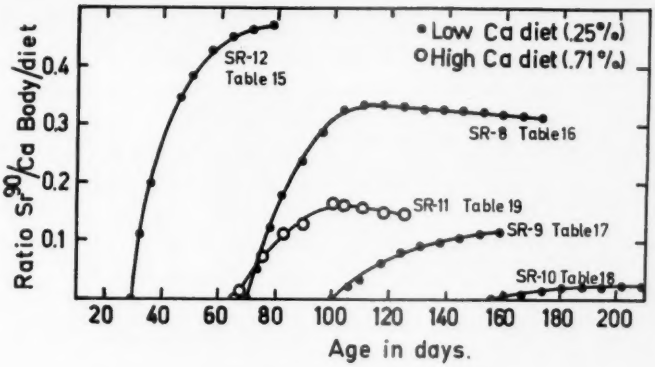

Figure 7

***The calculated Observed Ratio** (body/diet) in rats which have been on a continuous ingestion of Sr^{90} in the diet. The different experiments and the corresponding tables are indicated in the figure.*

principle, the same curve resulted for the rats in Sr-11, table 19. The effect of the high Ca diet is clear. The three other experiments on the 0.25 per cent Ca diet were not conducted long enough to result in the characteristic curve.

When next all four experiments conducted with the same level of Ca (0.25 per cent) in the diets are compared, the correlation to age is striking. The interpretation is readily at hand. The younger the rats, the higher is the rate of Ca absorption and consequently the Sr^{90} absorption. Furthermore, the older the rats, the greater is the pool of inactive body Ca with which the absorbed Sr^{90} is diluted.

The calculated value for the peak in Sr-8 (table 16), rests on the assumed value of 0.70 per cent for the total body Ca at the start of the experiment. An increase in the assumed value of 0.70 per cent body Ca will result in a movement of the calculated peak to the right and a decrease in the calculated O.R.'s (body/diet).The slope of the curve will become more negative. If, on the other hand, the value for the total body Ca is lower than the 0.70 per cent, the peak of the curve will move to the left, the O. R.'s (body/diet) will be higher, and the slope will become less negative.

B. The ratio of Sr^{90}/Ca in blood plasma

In the preceding experiments a direct estimate of the serum Sr^{90}/Ca could not be determined in the course of the experiments, but only at the end. It was desirable to obtain a more complete picture of the ratio Sr^{90}/Ca in the serum as related to time under experimental conditions identical to the balance studies.

Twenty-four rats of both sexes were taken into the experiment at the age of 46 days. The diet contained 0.266 per cent Ca and 0.4 per cent P; 13 μC Sr^{90} was added per kg diet of a nearly carrier-free solution of Sr^{90}-Y^{90} nitrates (1 mC contained 0.1 mg solids). The pooled serum from two rats was used for each determination.

The results appear from figure 8. The ratio of Sr^{90}/Ca reached constancy after about eight days on the experimental diet. COMAR *et al.* (1957), in man, and WASSERMAN *et al.* (1958) in goats, also found that serum ratios of Sr^{85} or Sr^{89} to Ca^{45} reached a constant level in the course of a few days, following daily ingestion of a given dose.

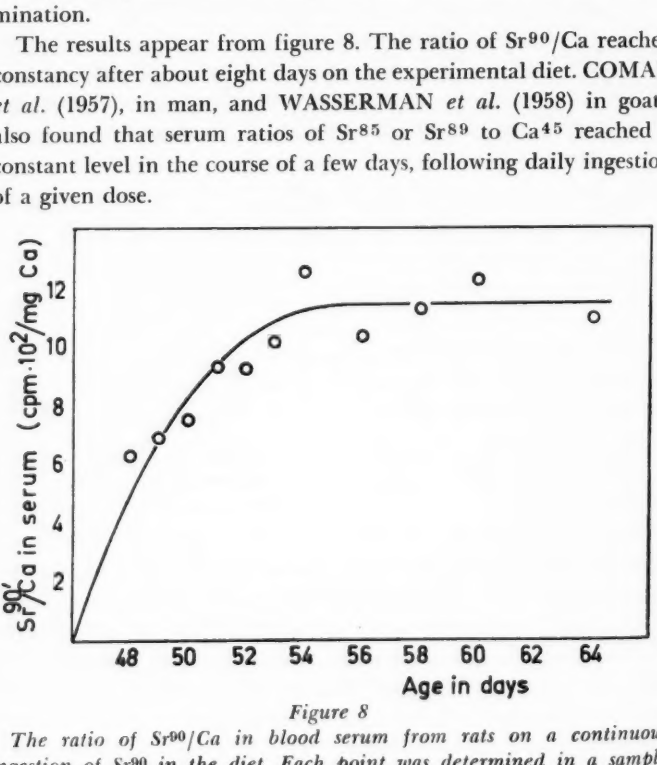

Figure 8

The ratio of Sr^{90}/Ca in blood serum from rats on a continuous ingestion of Sr^{90} in the diet. Each point was determined in a sample of pooled serum from two rats. The text should be consulted for further details.

Chapter 17

Observed Ratios (absorption)

In tables 15 to 19 the figures calculated for the Observed Ratios (absorption) are presented. The direct O.R.'s (absorption) have been calculated from the figures for the net absorbed Sr^{90} and Ca without any corrections for the amounts of Ca and Sr^{90} secreted with the digestive juices.

A second set of figures for O.R.'s (absorption), in which digestive juices Ca is corrected for, is also presented in the tables. Previously it has been shown that the figures for digestive juices Ca vary considerably (figure 6); however, an average figure of 15 mg DJ-Ca per day (tables 10 and 11) was used. The corrections have been carried out with the aid of equation 5 on page 29.

When the absorption of both elements was high (tables 15 and 16), the corrected figures did not deviate much from the direct figures. Corrected ratios smaller than the uncorrected resulted when the absorption of Ca and Sr^{90} was low (tables 16–19).

In one experiment (table 16) a correction has been applied for both Ca and Sr^{90} in the digestive juices. Equation 5, page 29, was used in order to obtain the corrected values for the absorption quotients. Sr^{90}/Ca was assumed to be secreted in the digestive juices in a proportion identical to the Sr^{90}/Ca in blood plasma. The figure for the Sr^{90}/Ca in serum (see footnote to table 16) was therefore applied. The figures for the O.R.'s (absorption) corrected in this manner were found to be in good agreement with the directly calculated O.R.'s (absorption).

The assumed value for DJ-Ca can in fact be varied considerably without affecting the corrected O.R.'s by more than about two per cent, provided that the utilization of dietary Ca is high. In the experiments presented in table 16, for example, the direct O.R. was calculated to be 0.81 in the second period. The values for the corrected O.R.'s were 0.81, 0.80 or 0.79, when 5,15, or 30 mg were used as figures for the daily DJ-Ca. In period 7, the corresponding O.R.'s were 0.56, 0.53, or 0.52, respectively, when the DJ-Ca was taken to be 5, 15, or 30 mg daily.

Other Observed Ratios

The values for the O.R.'s (body/diet) are found in figure 7. It is clear that these values are much lower than the values for the O.R. (absorption) because of the dilution by the pool of inactive Ca present in the body by which the absorbed Sr^{90} is diluted (table 21).

The average values for some other discriminative processes are presented in table 21. They represent the calculated averages for the balances in the experiment already presented in table 16. The O.R.'s determined at the end of this experiment are given in table 22. It appears that the O.R. (serum/diet) is in agreement with the calculated O.R. (body/diet). However, the distribution of Sr^{90} has not become uniform as indicated by the lower O.R.'s for (femur/diet) and (humerus/diet) than for (body/diet).

Table 21. The average figures for Observed Ratios in experiment Sr-8

Classification:	O.R.
Absorption/diet	0.57
Retention/diet	0.46
Body/diet	0.31
Urine/diet	3.75
Urine/serum	11.9

The figure are the averages for the experiment given in table 16.

Table 22. Observed Ratios at the end of experiment Sr-8

Classification	O.R.
Faeces/Diet	1.19 ± 0.02
Urine/diet	1.92 ± 0.06
Serum/diet	0.32 ± 0.05
Body/diet	0.31 ± 0.02
Femur/diet	0.25 ± 0.02
Humerus/diet	0.25 ± 0.02

The figures are the averages determined at the end of the experiment given in table 16.

Chapter 18.

The elimination of Sr^{90} from the body following discontinuation of Sr^{90} ingestion

In one of the experiments (table 17), the rats were continued on a non-radioactive diet in order to measure the excretion of Sr^{90} in

the urine and the faeces following discontinuation of Sr^{90} ingestion. Three days after the administration of Sr^{90} in the diet had been discontinued, the collections of urine and faeces were again started and the elimination of Sr^{90} was determined. The results are found in table 23.

At the beginning, a total of ten per cent of the dose retained during the last week of Sr^{90} ingestion was eliminated per day. The faeces were the more important route of excretion. The ratio of Sr^{90}/Ca in serum could not be determined in these rats while they were kept on the diet containing Sr^{90}. It was therefore necessary to compare the results in table 23 with indirectly calculated figures for the rats of comparable age in table 16.

The endogenous Sr^{90} in the faeces was calculated for the last period in table 16 in the following way: The calculations were carried out as described for Ca on page 28, assuming a daily secretion of 15 mg DJ-Ca and 894 cpm Sr^{90} per mg Ca. The endogenous Sr^{90} in the faeces was 11.9 per cent (in per cent of the 91.6 per cent). This figure corresponds to 0.47 per cent of the total body burden of Sr^{90} eliminated as endogenous Sr^{90} in the faeces per day. This is in fairly good agreement with the figure in table 23 of 0.33 per cent per day.

In the last period in table 16, the calculated body content of Sr^{90} was 836 cpm/mg Ca (table 20), compared to the net retention of 209 cpm Sr^{90}/mg Ca. The discrimination therefore results in the dilution seen in figure 7, (Sr-8).

Chapter 19

Double tracer experiments

Considerable variability in the calculated O.R.'s (absorption) resulted from the experiments presented in the previous sections. The values were correlated to the efficiency of the absorption of Ca, again dependent upon the age of the animal and the level of Ca in the diet. Additional experiments were therefore conducted in order to obtain accurate values for the Observed Ratios. The double tracer techniques as described by COMAR (1955) were used.

Three groups of rats were placed on a diet with 0.231 per cent

Table 23. Elimination of Sr^{90} in faeces and urine from rats which previously had been fed continuously on a Sr^{90} containing diet for 62 days
Experiment No. Sr-9

Days after discontinuation of Sr^{90} ingestion	Per cent excreted per day of total dose retained on the Sr^{90} ingestion		Per cent excreted per day of the dose retained in the last week of Sr^{90} ingestion	
	Urine	Faeces	Urine	Faeces
3rd to 5th day	0.20 ± 0.02	0.33 ± 0.02	3.8	6.2
5th to 7th day	0.17 ± 0.02	0.33 ± 0.02	3.1	6.2
7th to 10th day	0.15 ± 0.01	0.26 ± 0.02	2.8	4.9
10th to 21st day	0.05 ± 0.01	0.22 ± 0.01	1.0	3.9

Total elimination in 18 days, in per cent of total retained dose:

Urine	1.72 ± 0.13
Faeces	4.42 ± 0.17

The figures are mean ± standard error of the mean for six male rats, 162 days old when the administration of Sr^{90} in the diet was discontinued. Collections were started three days later. These rats have previously been reported in table 17, where the balance experiment for the radiostrontium feeding is given.

Ca and 0.38 per cent P containing 6 mg Ca^{45} and 54 mg Sr^{89} per kg diet. The diet therefore contained approximately 56 mg Sr/kg diet. The rats were 35, 74, and 270 days old, and received the diet two days ahead of the collections of urine and faeces. The actual experiment was conducted over four days. The rats were killed at the end of the experiment, and the blood from each group was pooled. The results are given in table 24. The O.R.'s (absorption) and O.R's (retention) were calculated from the balance data, and the other O.R.'s followed from the analytically established ratios in urine, serum, and diet, respectively.

The O.R.'s (absorption) in the two groups of very young rats were high, in fact even higher than those calculated in two earlier experiments, tables 15 and 16.

The explanation is probably the following: Nearly all Ca is absorbed; in rats 74 days of age only 1.3 mg Ca was excreted in the faeces daily. In consequence, practically all Ca^{45} ingested was also absorbed. Most of the 1.3 mg in the faeces may then be DJ-Ca secreted in the lower part of the gastro-intestinal tract, and a "falsely" high O.R. (absorption) will be the consequence.

Table 24. Double tracer studies with Ca^{45} and Sr^{89} in the diet

Age	Body weight	No. of rats	Ca, mg per day			Ca^{45} Per cent of intake		Sr^{89} Per cent of intake		Observed Ratios Sr^{89}/Ca^{45}			
days	g		intake	absorption	retention	absorption	retention	absorption	retention	absorption	retention	urine/diet	serum/diet
35	65	5	19.7 ± 0.8	17.0 ± 0.8	16.9 ± 0.7	92.7 ± 0.8	92.2 ± 0.8	71.5 ± 1.7	67.4 ± 1.7	0.77 ± .02	0.73 ± .03	7.2 ± 1.9	0.80
74	122	6	24.8 ± 1.2	23.5 ± 1.2	23.3 ± 1.2	99.8 ± 0.1	98.9 ± 0.1	88.0 ± 1.8	82.4 ± 2.0	0.88 ± .02	0.83 ± .02	5.6 ± 0.5	0.89
270	361	5	20.1 ± 3.3	0.5 ± 0.5	0 ± 0.5	40.3 ± 3.3	37.6 ± 3.4	12.2 ± 1.5	7.7 ± 1.7	0.30 ± .02	0.19 ± .03	3.1 ± 0.3	0.28

The diet used contained 0.231 per cent Ca and 0.38 per cent phosphorus. 6 mg Ca^{45} and 54 mg Sr^{89} was added per kg diet. The activities were approximately 320 cpm Ca^{45} and 460 cpm Sr^{89} per g diet.

The rats were started on the radioactive diet two days ahead of the collections of faeces and urine. Each experiment lasted for four days. The rats had previously been maintained on the pre-experimental diet with 0.25 per cent Ca and 0.4 per cent P; vitamin D was supplemented. Sera were pooled within each group for analyses.

Chapter 20

The absorption of Ca from a non-milk diet versus a milk diet

COMAR *et al.* (1956), LENGEMANN *et al.* (1957), and WASSERMAN *et al.* (1957) report striking increases in Ca absorption when milk diets were substituted for non-milk diets in man, rats, and cattle, but not in rabbits.

Such results, were they characteristic of the Ca absorption in general in milk diets in comparison with non-milk diets, would deeply affect the evaluation of the fall-out problem. In fact, milk being the one variable, curves like Sr-8 and Sr-11 would result (figure 7), Sr-8 representing the milk and Sr-11 the non-milk diet. The long-term result would also be correspondingly higher urinary Ca levels on milk diets in contrast to non-milk diets. In view of the importance of the problem, it was felt necessary to study the problem anew.

Two different diets were prepared where the Ca was supplied either as the carbonate or in the form of evaporated whole milk. The other dietary constituents of the milk diet were adjusted to give equal composition of the two diets with respect to total protein, fat, Ca, and P (table 2). A calculation of the amino acid content of the two diets shows that the milk diet was higher in its contents of arginine (9.5), cystine (13.4), iso-leucine (32.5), leucine (20.5), lysine (22.5), phenyl-alanine (10.2), threonine (19.0), and tryptophan (51.2). The higher figures for the milk diet are given in parentheses in per cent of increase. The non-milk diet contained 0.266 per cent Ca and 0.51 per cent P versus 0.253 per cent Ca and 0.57 per cent P in the milk diet.

A group of eight male rats approximately 250 days old was started on the milk diet two days ahead of the actual experiment on this diet. The rats had previously received the pre-experimental diet with 0.25 per cent Ca as well as 70 I.U. vitamin D_2 weekly for six months.

The absorption of Ca was studied for 22 days in four consecutive periods on the non-milk diet, and for 21 days in three consecutive periods on the milk diet. One day was allowed to pass without collections of faeces in the transitory phase.

The results are given in table 25. It is evident that in the experiment here presented, no effect was observed following transition from the non-milk diet to the milk diet with identical Ca levels (the calculated p was > 0.75).

Table 25. The absorption of calcium from milk and non-milk diets

Period No.	Diet	Duration days	Body weight g	Change in body weight g	Calcium intake mg/day	Calcium absorption mg/day
1	$CaCO_3$	4	354	+ 2	33.4 ± 1.8*	7.2 ± 0.8
2		4	356	— 4	34.1 ± 1.8	7.4 ± 0.7
3		7	352	0	37.3 ± 1.2	6.9 ± 0.4
4		7	352	— 4	36.5 ± 1.2	6.3 ± 0.4
Weighted mean for 22 days on diet with $CaCO_3$					35.8 ± 0.9	6.8 ± 0.4
5	Milk	7	348	+11	36.9 ± 1.0	6.7 ± 0.5
6		7	359	+ 1	39.1 ± 1.1	8.8 ± 0.8
7		7	360	+ 4	36.5 ± 1.2	5.8 ± 0.6
Weighted mean for 21 days on diet with milk					37.5 ± 0.6	7.1 ± 0.7

* Standard error of the mean.

The data are averages from eight male rats approximately 250 days old when started in the experiment. The rats had been fed on a diet containing 0.25 per cent calcium with 70 I.U. vitamin D per week for the last six months before they were started on the experimental diet.
Serum calcium was 10.9 ± 0.2 mg per 100 ml at the end of the experiment. The $CaCO_3$ diet contained 0.266 per cent Ca, the milk diet 0.253 per cent Ca.

It is of interest to observe that COMAR *et al.* (1953) state that according to unpublished data no difference "in the absorption of Ca^{45} from milk as compared with that of $Ca^{45}Cl_2$" had been observed.

In the course of the last decades a great number of observations on the effects of various factors on the absorption of Ca have been published, and many conflicting results have been reported. In view of the very fine regulation of Ca metabolism, the effect of vitamin D, of adaptation, etc., it is not difficult to see how various and conflicting results may be obtained from short-term experiments. The subject has recently been well reviewed by IRVING (1957). The experiments of the LEICHSENRING-PATTON group (LEICHSENRING *et al.* 1951, PATTON *et al.* 1953) are

illuminating. The conclusion in the first paper is that additional P in the diet depressed Ca absorption, in the second that no such effect could be observed. The experiments of NICOLAYSEN (1943) on the effect of fatty acids on Ca absorption, of NICOLAYSEN and NJAA (1953) on the effect of phytate, of LOVELACE, LIU, and McCAY (1950) on the effect of oxalate, and numerous other experiments (e.g. MALM 1958), indicate how adaptation of Ca absorption deeply affects the results in Ca balance studies.

In view of the previously presented experiments it is not to be expected that O.R. (absorption) will be materially altered when the Ca absorption remains constant.

Chapter 21

General Discussion

The absorption of Sr^{90} is reduced when the dietary supply of Ca is in excess of the requirements (tables 16 and 19). WASSERMAN *et al.* (1957) have previously shown that additional Ca in the diet reduced the retention of Sr^{89} and Ca^{45} in the same proportion. In their experiments the lowest level of dietary Ca was 0.5 per cent. A reduction of the dietary Ca to 0.25 per cent, which is adequate for the growing rat, resulted in higher values for the O.R.'s (absorption) in the experiments here presented, because of a complete absorption of Ca in the upper part of the small intestine.

The age of the rats *per se* apparently affects the O.R. (absorption) only as far as age affects Ca absorption. The results obtained are in line with this contention. The results of COMAR *et al.* (1955) showed that the O.R. (body/diet) was approximately identical when groups of rats of different ages were compared. Their rats had received a diet containing Sr^{90} with 1.9 per cent Ca over the lifetime. Other results from the same laboratory indicate, however, that the O.R.'s (bone/diet) in cattle and rats decrease with increasing age (COMAR and WASSERMAN 1956, 1957). These experiments were carried out by single dose administrations.

The age of the rats at the time of the start of Sr^{90} ingestion influences the pattern of Sr^{90} accumulation in the body. In addition to the low rate of Sr^{90} absorption in the older rats, the absorbed Sr^{90} is diluted with a larger body pool of uncontaminated Ca. The combination of these two factors results in lower

figures for the O.R.'s (body/diet) in the older rats. The peak of the curve (figure 7) was not reached in exp. Nos. Sr-9 and Sr-10; these rats will therefore slowly increase their body ratio of Sr^{90}/Ca. The curves are almost horizontal, however, which indicates that the additional time required to reach the peak is considerable. It is conceivable that the oldest rats actually may never reach the peak during their lifetime.

The characteristic curves for the O.R.'s (body/diet) presented in figure 7 are to a large extent self-explanatory. Some comments are needed on the decrement of the O.R.'s (body/diet) ensuing upon the peak of the curves in Sr-8 and Sr-11. An explanation offers itself. At the peak, Sr^{90}/Ca "balance" or "equilibrium" between the body and the diet has been established. Next, Ca is retained and accreted in excess of Sr^{90} because of the discrimination against Sr^{90} in the reabsorption of DJ-Sr^{90} in the intestine, and in the kidneys. This seems to be another type of dilution effect which will continue to reduce the Sr^{90}/Ca ratio in the body until the optimal adult skeleton is formed. It must be expected that in the end the curve will become parallel to the abscissae as the rates for skeletal accretion and resorption decrease.

The combination of a low Ca diet and very young, rapidly growing animals favoured the development of an O.R. (body/diet) very near to that maximally achievable on any diet adequate in Ca. In fact the Ca absorption was complete in the early periods of experiments in the very young rats.

Experimental results previously quoted indicate that sizable amounts of stable Sr in the diet could interfere with the metabolism of Sr^{90}. However, the amounts of stable Sr in the diets were very small (see page 65), and the very high Sr^{90} absorption in these young rats strongly disfavours the possibility of an actual interference. In fact the highest O.R. (absorption) observed in the experiments here presented are much higher than practically any previously reported values (cf. UNITED NATIONS SCIENTIFIC COMMITTEE 1958), though the possibility remains that the lower ratios found by most other workers may be explained by higher contents of stable Sr in their diet. The double tracer experiments carried out here, however, indicate that the same ratios are obtained when the stable Sr content of the diet is many times increased.

It is inconceivable that the stable Sr content of the skeleton could affect the absorption of Sr^{90} in any other way than does skeletal Ca. The experimental evidence discussed previously indicates that there is no discrimination against Sr in the transfer from blood plasma to the bones. It may be of interest to future workers in the field to know that stable Sr analyses of the whole skeleton gave 380 micrograms Sr per g Ca in 43-day-old rats which had received the pre-experimental diet for 17 days.

Next it may be of interest to discuss shortly the findings on Sr/Ca in human bones. Analyses of the stable Sr/Ca ratio in human bones have resulted in somewhat higher values for adults compared with children (HODGES *et al.* 1950, TUREKIAN and KULP 1956, SOWDEN and STITCH 1957, THURBER *et al.* 1958, and ALEXANDER and NUSBAUM 1959). ALEXANDER and NUSBAUM (1959) also give values for the Sr/Ca ratio in milk and vegetables; the higher values found in the latter may suggest that the dietary ratio of Sr/Ca increases as the children gradually adopt the dietary habits of the adults. This change in the dietary Sr/Ca ratio may explain, at least in part, why higher values for the Sr/Ca ratio are found in adult skeletons.

The findings of later years show that the Sr^{90}/Ca is highest in children (KULP *et al.* 1957, ECKELMANN *et al.* 1958); the maximum values are found in children less than three years old (KULP *et al.* 1959). It is not known how close this age group is to the establishment of a constant Sr^{90}/Ca ratio in their bodies, provided the Sr^{90}/Ca in the diet had remained constant. The continuous increase in the Sr^{90} contamination of our diet in recent years suggests that even this age group may be far from the peak. On the other hand, KULP *et al.* (1959) give figures which allow a calculation of the O. R. (bone/milk) for the group 0–3 years old. This ratio was 0.22 (1 July 1957) which is close to the ratio of stable Sr/Ca found in adult humans (BRYANT *et al.* 1958) of 0.25. Moreover, 25 per cent or less of the Ca in the skeleton of a two-year-old child, previously breast-fed for half a year, will have been derived from the maternal milk, and in addition 15–20 per cent of the bone Ca is derived from the mother during gestation. However, the possibility remains that children may parallel our rats because of the relatively high efficiency of Ca utilization.

One way to investigate this problem is the following. The find-

ings of COMAR *et al.* (1957 b), WASSERMAN *et al.* (1958) as well as in this work indicate that a constant level of Sr^{90}/Ca is rapidly established in the blood plasma when Sr^{90} is continuously ingested. The O.R. (plasma/diet) was found to be equal to the O.R. (body/diet) after the peak had been established (figure 7). Analyses of Sr^{90}/Ca in human plasma may therefore be of great importance. Sufficient blood may be obtained from autopsies or by pooling discarded blood bottles from the blood banks. The results will indicate the "equilibrium" level of Sr^{90}/Ca at the present Sr^{90}/Ca level in the diet.

It is extremely important in view of the present situation to establish for humans curves resembling the ones presented in this work on rats.

A theoretical curve for the increase in dose-rate to the critical organ as a result of continuous ingestion of Sr^{90} has been calculated by the INTERNATIONAL COMMISSION ON RADIOLOGICAL PROTECTION (1954) for an adult standard man. This curve cannot be applied to our present problem of skeletal accumulation in humans of Sr^{90} from radio-active fall-out because of the following facts:

a) A constant biological half-life has been assumed. The biological half-life obviously is dependent upon the rates of skeletal turnover, which will decrease with increasing age.
b) It has been assumed that a constant fraction of the ingested dose reaches the skeleton. This fraction is dependent upon the state of Ca absorption which changes with the age.
c) The mass of the critical organ was assumed to be constant. The size of the skeleton increases in growth. Later in life a decalcification of the skeleton starts.

These comments also apply to the theoretical curve of NORRIS, TYLER, and BRUES (1958). It is evident from the actual curves found in growing rats that the skeletal Sr^{90}/Ca reaches a maximum very rapidly (figure 7).

The best protective measures against an excessive accumulation of Sr^{90} in the human skeleton appear to be a liberal Ca supply in the diet. This is of special importance in the periods of life when the rate of Ca absorption is high, and in the regions where the daily Ca intake is low. It may be more questionable if a Ca supplementation is necessary in countries where the daily Ca intake is

high. In Norway, for example, the average family consumes between 30 and 40 mg Ca per 100 calories, with a tendency towards higher figures in families with small children (ØGRIM, personal communication). Approximately 80 per cent of the dietary Ca derives from milk and dairy products in this country. On the other hand, Norway seems to receive more radioactive fall-out than most other countries (HVINDEN 1959), and the Sr^{90} content of the milk is high compared with other countries (BERGH *et al.* 1959).

Part III

THE ACCRETION AND RESORPTION OF CALCIUM IN THE SKELETON

A. Introduction

The biochemical dynamics of the skeleton are discussed by NEUMAN and NEUMAN (1958) in their excellent book. The growth of the skeleton involves an accretion of bone mineral as well as a remodelling whereby the characteristic shapes of the bones are maintained. The last process involves a resorption of bone mineral already deposited. The accretion and resorption continue in the mature skeleton and are normally in balance. A fresh supply of new and reactive bone is thereby maintained.

After the administration of a single dose of a bone-seeking radio-isotope, the disappearance curve from the blood plasma is determined by the rates for ion exchanges with the extracellular fluids and the bone salt, bone accretion, and the excretions via the kidneys and the digestive juices. Equations containing four exponential terms have been developed for the time/concentration curve of Ca^{45} in blood plasma by THOMAS *et al.* (1952) and ARMSTRONG *et al.* (1952). Simplified mathematical equations have been developed by BAUER, CARLSSON, and LINDQUIST (1955 b), and MIGICOVSKY (1957). They disagree, however, in their interpretation of the results with regard to the mode of action of vitamin D. A criticism of the approach of BAUER *et al.* (1955 b) is given by NEUMAN and NEUMAN (1958).

NEUMAN and NEUMAN (1958) suggest that attempts to solve these problems should be directed towards experiments where the concentration of the isotope is kept at a constant level in the plasma. In the experiments here presented, it has been found that the specific activity of Ca^{45} in plasma, as indicated by urine analyses, will reach a constant level in the course of two to three days when a diet with a constant specific activity is given to adult rats adequately supplemented with vitamin D. The plasma ratio of Sr^{90}/Ca was constant after approximately eight days in young, rapidly growing rats (figure 8). In adult, vitamin D free rats, the

plasma concentration of Ca^{45} increased slowly, as shown in figure 4.

B. Calculations

Retention of Ca^{45} by ion exchange will be negligible provided time is allowed for the equilibration between the exchangeable part of the bone mineral and the plasma. The small uptake of Ca^{45} which is caused by the slow intracrystalline exchange is disregarded.

The increment in the skeletal content of Ca^{45} during a metabolic period is expressed by:

$$Ca^{45}_{retained} = Ca^{45}_{accreted} - Ca^{45}_{resorbed} \qquad \text{(Eq. 1)}$$

The last term of this equation will most probably be small if the measurements are taken shortly after constant activity has been obtained in the plasma, and it is therefore excluded in the primary calculation. An error is obviously introduced, resulting in too low values for accretion.

The constant activity of the plasma is the result of a continuous mixing in the blood plasma of the following components: Ca^{45} and inactive Ca absorbed from the intestine, and stable Ca from resorbed bone.

The figures from the balance studies furnish the necessary values for the calculations. The amount of Ca accreted in the metabolic period is now readily calculated:

$$Ca_{accreted} = \frac{Ca^{45}_{retained}}{\text{spec. act. plasma}} \qquad \text{(Eq. 2)}$$

The resorbed Ca is then given by:

$$Ca_{resorbed} = Ca_{accreted} - Ca_{retained} \qquad \text{(Eq. 3)}$$

C. Results

This method of calculation has been applied to the balances from some experiments carried out in this work. A selection has been made among the experiments in order to correlate the accretion and resorption of Ca to the size of the skeleton. The same principles of calculation have also been applied to one experiment

Table 26. Calculated rates for bone accretion and bone resorption in rats

Group	Group No.	Age days	Body weight g	No. of rats	Isotope	Days on isotope administration	Spec. act. serum % of diet	% of isotope retained	Ca-retention mg/day	Ca accretion mg/day	Ca accretion mg/day /100 g b.wt.	Ca resorption mg/day	Ca resorption mg/day /100 g b.wt.
70 I.U. vit.D weekly	1	35	65	5	Ca^{45}	2— 6	21.0	92.1	16.9	38.0	58.5	21.1	32.5
	2	74	122	6	«	2— 6	51.0	98.9	23.3	48.8	40.0	25.5	20.9
	3	78	170	6	Sr^{90}	8—12	33.4	66.4	24.2	52.0	30.6	27.8	16.4
	«	96	214	6	«	20—24	33.4	56.2	26.3	52.0	24.3	25.7	12.0
	4	180	383	6	Ca^{45}	5— 9	31.4	29.9	3.2	34.3	9.0	31.1	8.1
	5	270	361	5	«	2— 6	36.0	39.1	0	21.7	6.0	21.7	6.0
*	6	590	297	6	«	2— 6	41.9	76.4	17.7	50.0	16.8	32.3	10.9
	«	598	296	6	«	11—14	41.7	46.5	10.1	34.7	11.7	24.6	8.3
No vit. D	7	726	229	4	«	10—14	7.0	15.3	—7.0	16.5	7.3	23.5	10.4
	«	734	227	4	«	22—26	11.0	16.8	—6.0	18.8	8.3	24.8	10.9

* Group 6 received vitamin D 14 days before the start of the experiment for the first time in 6 months.

carried out with Sr^{90} (table 16, Exp. No. Sr-8). The figures are given in table 26.

The highest rates (mg/ 100 g body weight) for both accretion and resorption were observed in the youngest groups, as would be expected. Within limits, the body weight may be taken as an expression of the skeletal content of Ca (SHERMAN 1947). The figures obtained with Ca^{45} and Sr^{90} respectively agreed remarkably well.

The figures for Ca retention indicated that the rats given vitamin D shortly before the actual experiment (table 26, group 6) adapted as expected (NICOLAYSEN 1956). In consequence the skeletal accretion is about twice as high as in adult rats adequately supplemented with vitamin D (table 26, groups 4 and 5). The Ca resorption rate seems to be relatively unaffected. In group 6, Ca retention decreased to about one half of the initial figure in the last of the periods of observation, and is reflected in a reduced rate for Ca accretion.

In the vitamin D free rats, increasing activities of Ca^{45} in the urine were observed (figure 4). Next, the Ca accretion and resorption were calculated with the aid of the specific activity of the urinary Ca (assumed to equal the average plasma Ca specific activity in the actual period of experiment). In table 26 the values for this group indicate a subnormal accretion in contrast to the apparently normal resorption.

D. Discussion

The exclusion of the last term in equation (1), page 92, must result in estimates for $Ca_{accreted}$ and $Ca_{resorbed}$ which are too low, since $Ca^{45}_{resorbed}$ actually should have been added to $Ca^{45}_{retained}$ in equation (2), page 92. The figures for $Ca_{accreted}$ and $Ca_{resorbed}$ calculated (table 26) by the method will therefore be the minimal values. The substitution of varying values for $Ca^{45}_{resorbed}$ in equation (1) will result in increments of the calculated values for accretion and resorption, and these increments will be numerically equal. With increasing error due to the omission of $Ca^{45}_{resorbed}$, the values for Ca accretion and Ca resorption will increase linearly, as shown in figure 9.

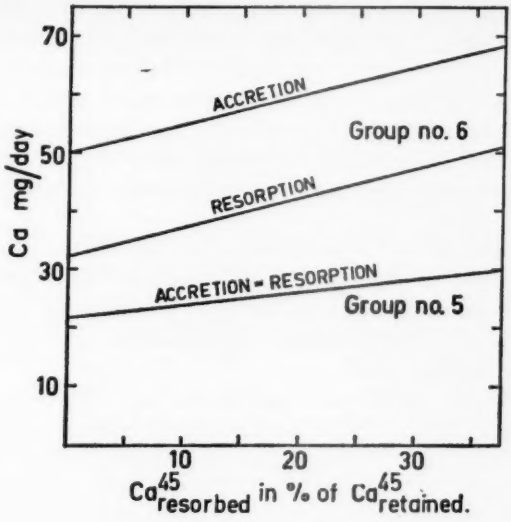

Figure 9

The straight lines represent the values resulting from the substitution of a set of values for $Ca^{45}_{resorbed}$ in equation (1) page 92. Table 26 should be compared.

The results here presented indicate, as expected, that accretion and resorption balance each other when maturity has been reached. When the rate of growth is high the accretion, as foreseen, is the dominant process. However, the values for resorption, in fact below the true ones, are remarkably high. It may be of interest to note that a twenty per cent error will result in a value for resorption of 38.2 mg in group 3, period 1. The corresponding value for accretion is obviously also increased by 10.4 mg.

BAUER, CARLSSON, and LINDQUIST (1955 a, b) found that the rate of accretion was 65 mg Ca per day in rats with approximately 1,000 mg body Ca. In other experiments, BAUER *et al.* (1956) reported that 58 mg Ca per day was accreted in rats of comparable size. These values are in good agreement with groups 2 and 3 in table 26.

The Swedish group (BAUER *et al.* 1955 b) has concluded that a primary effect of vitamin D is to promote bone resorption. The results here presented do not seem to support such a contention.

When group 6 is compared with groups 4 and 5 (table 26) it appears that the chief difference is that accretion is substantially higher in the rats which only recently received vitamin D. The values for resorption are about the same in the groups. Figure 9 indicates that when errors are corrected for, the conclusion is still equally valid. As previously mentioned MIGICOVSKY (1957) also produced evidence that was not in accord with the Swedish point of view.

MIGICOVSKY (1957) pointed out that higher rates of accretion in the rapidly growing animal are associated with high rates of resorption due to the rapid remodelling of the bones. The figures arrived at in the experiments here presented are fully in line with such a contention.

Vitamin D induces increased plasma Ca independent of the improved absorption following the administration of this vitamin (CARLSSON 1952, NICOLAYSEN and EEG-LARSEN 1956). An increment of 3 mg Ca per 100 ml plasma corresponds to about 0.8 mg Ca in the extracellular fluid in an adult rat of 300 g body weight. Such a value is a very small one in comparison to the daily resorbed amount of Ca.

The possibility remains that high rates of resorption may occur immediately after the administration of vitamin D. The action of the parathyroid glands on the bone resorption may be a possible explanation of such an effect. The glands are usually hypertrophic in vitamin D deficiency, indicating an increased production of hormone. When next vitamin D is given, the combined effects of the vitamin and the hormone result in an initial increase in the bone resorption.

Summary

A method is described for the determination of digestive juices Ca, based on the continuous ingestion of Ca^{45} with stable Ca in the food. The true absorption of Ca can next be estimated.

In the adult rat the digestive juices Ca was found to be in the range of 6.4 to 27.6 mg daily. The variability was considerable. When a number of consecutive periods were averaged in four vitamin D free rats, the variability was about $\pm$ 15 per cent of the mean value of 11.2 mg Ca daily.

Variations in serum Ca concentrations influence the digestive juices Ca as expected. When ionized Ca was calculated, a straight line relation was found between the ionized Ca and the amount of digestive juices Ca.

Variations in the amount of food eaten resulted in proportional variations in the digestive juices Ca in rats supplied with vitamin D. In vitamin D free rats, variations in voluntary food consumption resulted in corresponding changes in the secretion of Ca with the digestive juices, not, however, to the same extent as in the rats supplemented with vitamin D.

The method was found to be applicable with small errors only when the speed of Ca absorption was low, Serious errors may readily occur when the rate of Ca absorption is high or when high Ca diets are used.

Some experiments in which the isotope was administered in a single dose by stomach tube revealed the serious weakness of such a procedure in contrast to the continuous feeding administration.

Long-term balance experiments were carried out to study the accumulation of Sr^{90} in rats with a constant level of Sr^{90} in their diet. Four groups were taken into experiment at different ages on a diet with 0.25 per cent Ca. A fifth group received a high Ca diet (0.71 per cent). The rats were kept in the experiment for 50 to 103 days.

In the youngest group of rats the net absorption of Ca was nearly complete. The rats on the 0.71 per cent Ca diet did not absorb more Ca than rats of comparable age on the 0.25 per cent diet. The Ca absorption decreased with increasing age of the rats, as expected.

Sr^{90} in faeces increased in the course of the experiments; the initial figures for faecal excretion in each experiment were definitely correlated to the efficiency of Ca absorption. An increase in the level of Ca in the diet resulted in a proportional depression of Sr^{90} absorption.

The Observed Ratio (absorption) was calculated from the figures for net absorption of Ca and Sr^{90}. Corrections have also been made for the secretion of Ca and Sr^{90} with the digestive juices. The O.R. (absorption) was found to be very high in the youngest rats, which is explained by the very high utilization of dietary Ca in the rats on the 0.25 per cent Ca diets.

The urinary level of Ca was low in all the experiments. Sr^{90} in the urine remained within ten per cent of the intake in practically all observations. There was a correlation between Sr^{90} absorbed and the urinary excretion. Within a given experiment, the Sr^{90} level in the urine became constant from the very early periods.

The ratio of Sr^{90} in serum became constant after about eight days in young rats on continuous ingestion of Sr^{90}.

The Sr^{90}/Ca ratio in the body was calculated at the end of each metabolic period. The resulting curves show a peak after approximately 33 days on the Sr^{90} diet. Next, some decrease followed, due to absorptive and urinary discrimination. The highest ratios for Sr^{90}/Ca (body/diet) were found in the youngest rats, corresponding to the highest rates of absorption and the smallest body pool of Ca. The ratio of Sr^{90}/Ca in the body was reduced in proportion to the increase of Ca in the diet.

Following discontinuation of Sr^{90} ingestion, the faecal excretion was about twice as high as the urinary elimination.

The figures for Ca and Ca^{45} retention were used in a calculation of the rate of bone accretion and resorption. The plasma Ca specific activity was constant in the periods used. The calculation rested on the presumption that the specific activity of Ca in accreted bone equals the specific activity of Ca in the plasma. In the first approximation zero specific activity of resorbed Ca was

somewhat erroneously assumed. However a calculation with the aid of a set of figures for the specific activity of resorbed Ca results in: a) that accretion and resorption show a parallel increase, and b) gives a fairly good estimate of the probable size of the two variables under various experimental conditions. Experiments with vitamin D indicate that the vitamin acts primarily by increasing Ca absorption and bone accretion.

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